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CELLULAR CHANGES IN THE CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH HERPES SIMPLEX AND VACCINIA

A Study with Thin Sections for the Electron Microscope*

FREDERIK B. BANG

With the Technical Assistance of Fred Ise

Department of Medicine, Johns Hopkins University School of Medicine and Hospital

Received for publication July 24, 1950

Since most plant and animal viruses are too small to be seen with the ordinary microscope, the electron microscope has yielded new information about a number of them (1, 2). Some have been studied in tissue culture (3–5) but there has been no consistent attempt to study the development and growth of animal viruses by observing the practicable thin sections (6–8) prepared currently for the electron microscope. Exceptions to this are the virus of tobacco mosaic which has been seen in sections of leaf cells of tobacco plants (9), and the virus of fowl pox which has been studied in thin sections of the chick chorio-allantoic membrane (10).

METHODS

Strains of Virus Used

Herpes simplex, the H.F. strain which had been passed through chick embryo passages was received by us from Dr. T. F. McNair Scott and has subsequently been through two embryo passages.

A second strain was obtained by inoculating chick chorio-allantoic membrane with fresh vesicle fluid from a young adult female who had a series of fever blisters at the corner of her mouth. This material on embryo passage was sterile when tested in ordinary bacteriological media. The second embryo passage was used in this study. Typical intranuclear inclusions were apparent in both of the above strains in Zenker's fixed chorio-allantoic membrane stained with hematoxylin and eosin.

Vaccinia. A standard strain of this virus was obtained from a commercial (Wyeth) calf lymph vaccine and was inoculated on the chick chorio-allantoic membrane and transferred twice thereafter before being used in this study.

* Supported in part by Grants in Aid from the Damon Runyon Fund and from the National Institutes of Health, U. S. P. H. S.

Presented in part to the Society of American Bacteriologists May 15, 1950.

The fourth virus strain studied was isolated from a child with disseminated vesicular lesions which were diagnosed clinically as Kaposi's varicelliform eruption. This agent, when inoculated on rabbit corneas, produced keratitis and subsequent conjunctivitis in one to two days. No neurological lesions resulted. No skin lesions were apparent after inoculating the freshly shaved skin of two rabbits. On the chorio-allantoic membrane of chick embryos 11 to 13 days old it produced scattered pock-like lesions at dilutions of 10^{-4} and 10^{-5}. Smear preparations from the infected chick chorio-allantoic membrane, examined under the electron microscope showed brick-shaped particles of the same size as vaccinia.

Fixation

Except for a few preliminary studies on fixation methods, our material has been uniformly prepared by dropping small pieces of chorio-allantoic membrane, immediately after cutting it from the whole membrane, into 2 per cent osmic acid solution This tissue remains therein for periods of 2 to 24 hours when it is thoroughly rinsed with distilled water and passed through the alcohol series up to the methacrylate embedding procedure outlined by the Bureau of Standards workers (7).[1] Most of our studies have been carried out with unshadowed thin sections contained in the final state in a collodion membrane prepared from an amyl acetate solution. Such membranes are particularly likely to break if the microscope beam becomes too intense. Coating the membrane with chromium increases the resistance of the membrane and highlights any projecting portions of the specimen. However, it obscures a number of important details. We have coated a number of sections with chromium but in the study of herpes have gained no new information thereby.

RESULTS

A comparison of Zenker's, neutral formalin, Carnoy's and osmium fixation rapidly showed that osmium fixation is far superior to the other three, as has been reported for tissue culture (11, 12) and sections of other cells (6, 8). This has been true in our hands not only for the normal cell but also for cells infected or destroyed by herpes, Newcastle virus or eastern equine encephalomyelitis. The heavy rope-like precipitate formed, particularly with Zenker's and Carnoy's, makes it impossible to study cellular detail or to differentiate one virus particle clearly from another or from the background. Thus, our description of the normal cells of the chorio-allantoic membrane will be limited fairly strictly to osmium-fixed material.

Visible in Fig. 1 is a portion of an epithelial cell in which the sec-

[1] We are indebted to Drs. Newman, Borysko and Zwerdlow for learning their technique from them firsthand.

tion was made through the center of the nucleus. This may be compared with Fig. 2 where only an edge of the nucleus is within the section. In both the cells certain parts are clearly distinguishable. There are mitochondria varying in width from about 100 mμ to 200 mμ and apparently branching at several places. These are generally homogenous and in the normal cells show no evidence of a limiting membrane. Other sections frequently show the association of a fat droplet with a mitochondrium, and this seems to be the case at the upper end of the long mitochondrium in Fig. 1. This has also been noted in tissue culture studies of certain fibroblasts (13). A fine reticular background substance is present throughout the cell and as an integral part of this structure are irregular particles varying in size from 10 mμ to 100 mμ. This lower dimension is about the limit of resolution which we have been able to attain with sectioned embedded material and it is possible that particles of smaller size are also present. No clear image of a cell membrane is apparent.

The nucleus is surrounded in Fig. 1 by a thickened condensation of opaque material; its irregular distribution in Fig. 1 and its apparent absence in Fig. 2, suggest that much of the thickness of this membrane is a mechanical artifact of fixation. The nucleus shows a fine granular appearance with the large dense nucleoli usually occupying a slightly eccentric position. No details of nucleolar structure are apparent.

Some visualization of cellular structure is possible in these same epithelial cells grown in tissue culture and Fig. 4 is a picture taken near the edge of such a cell. In general, the resolution is poorer.

The loose mesenchymal tissue between the ectodermal and entodermal layers is made up of scattered fibroblastic cells held together by a multiplicity of intertwining fibers. These have a periodicity in more cellular areas of about 50 mμ and a width of the same throughout their extent, which corresponds roughly to that of collagen (14). However, a striking variation in periodicity from 50 to 135 mμ is seen in one array of fibers connected to cells as illustrated in Fig. 3. The apparent stretching of some of these fibers, which are thinner and have longer periodic intervals when extended between cells, raised the question as to the natural state of these fibers in the living tissues.

The presence of such a series of graded intervals suggests that the

fibers are naturally extended within the cell and that the intervals become apparent as the extended portions of the fibers contract into globular masses which then remain connected in a series.

Changes Apparent in Infected Cells

Herpes Simplex (*Strain HF*). The outstanding changes seen in our material are in the cytoplasm of the cell. There is a distension of the cytoplasm, an enlargement, thickening, and finally a vesiculation of the mitochondria. Often these organelles become plastered on the surface of the nucleus so that with less optical resolution it would be difficult to separate them from the nuclear membrane. Scattered throughout the cytoplasm and also apparent within some nuclei are dense osmiophilic particles occurring occasionally in clumps, and smaller than the usual estimate of 150 mμ for the size of this virus (15). Around these may be seen an irregular accumulation of material. It is not possible to identify these particles as virus, nor have we found any other consistent particles within the cells.

Strain MF—The changes brought about by this strain are very like those made by the classical strain, though the vesiculation and disintegration of the mitochondria may be even greater. With strain MF, pieces of the walls of mitochondria are often seen lying free within the cytoplasm. As with the other strain, it has not been possible to identify the virus with certainity. However, in a number of sections roughly brick-shaped particles with small dense centers may be seen. These centers correspond in size to the scattered dense particles seen in the H.F. strain.

Vaccinia—Most of the observations here described and illustrated were carried out with the virus recovered from the patient with varicelliform eruption. However, one series of sections from a chick chorioallantoic membrane infected with the known strain of vaccinia are so similar that the description may be applied to both. In startling contrast to the sections of herpes, particles which can possibly be identified as virus appear in these sections. They are so identified because of their characteristic shape, frequency and close similarity to the particles seen in purified preparations of the virus (16, 17). Unlike the purified preparations or smears of the infected membrane, there is considerable variation in size among these particles (Chart 1). This

may be due in part to the fact that the particles in a section have different orientations. It may also be that the sections actually show

SIZE DISTRIBUTION OF "VIRUS" PARTICLES AS SEEN IN SECTIONS

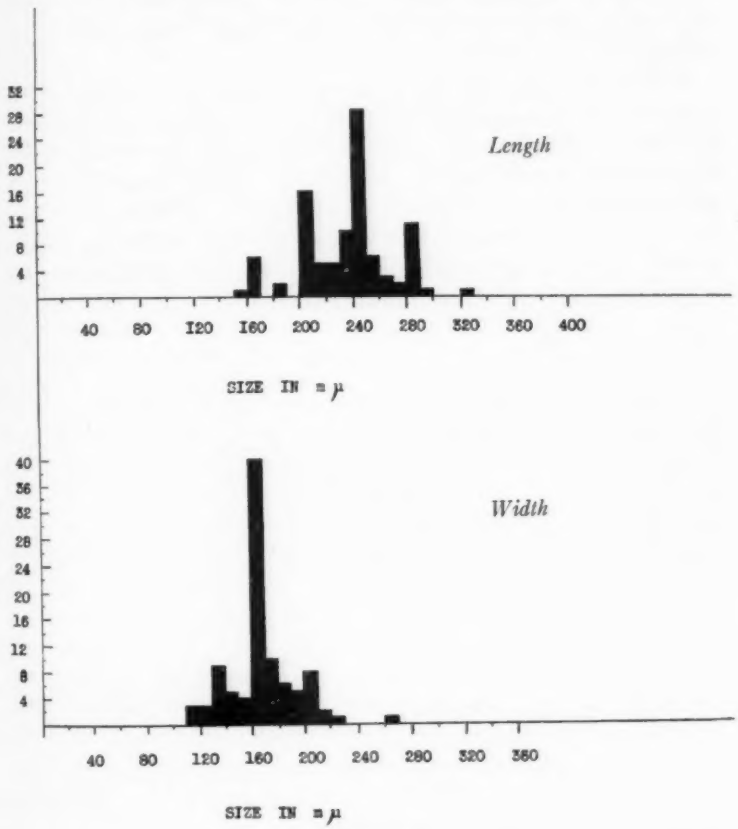

CHART 1

only fragments of the virus, for a section 0.1 micron thick would not be as thick as many of the longer particles.

One remarkable finding is the number and frequency of these clusters of particles. They are not always scattered uniformly throughout the cell but often stick together. The mitochondria have for the most part

disappeared and indeed the relationship of these groups of virus particles to the occasional intact and recognizable mitochondrium is suggestive of a close relationship between virus and mitochondrium (Figs. 14, 15). A similar observation was made on infected epithelial cells in a tissue culture (Fig. 16), prepared and studied by Dr. J. S. Murphy.

The heaviest infection found in the sections was in cells near or lining the blood vessels and thus extending deep into the mesoderm. However, in epithelial cells, which probably came from the entodermal layer of the chorio-allantoic membrane, exposed fringe-like edges showed dense particles extending into the individual blunt projections. The appearance of these apparent virus particles within such stalks is very similar to the occurrence of vaccinia virus particles in cytoplasmic filaments extending from epithelial cells in tissue culture (18).

DISCUSSION

In the past, theories concerning the growth and/or increase in infectious virus units in association with cells have been dependent upon biological and biochemical data. Such theories have forged ahead of morphological data concerning the presence of the virus in different parts of the cell or indeed data as to whether a particular virus actually existed in the cell or was merely tightly bound to it. The increased resolution of the electron microscope over the ordinary microscope has brought about direct visualization of many biological structures one tenth the size of those previously visible. Thus, we can confidently expect to see particles as small as the virus of poliomyelitis. Identification of the various particles is, however, a profoundly difficult step. Since such viruses do not grow synthetic media, it is logically impossible to identify any particle as the etiologic agent of a disease. So identification of viruses has reached the frontier of knowledge occupied by leprosy, malaria and rickettsial diseases.

In the sections of herpes, we have been unable to identify particles as virus with any certainty. In contrast, sections of material infected with vaccinia or the agent obtained from a child with varicelliform eruption contained such masses of characteristic particles that identification of these becomes much more likely.

The type of fixation and the embedding and cutting procedures used

in electron microscopy are being investigated in many places. Our preliminary trials with the double embedding procedure of Baker and Pease (6), as modified by Hillier and Gettner (8), has not as yet yielded as satisfactory results as the plastic embedding method (7).[2] However, the latter method is not free of artifacts. Scattered perfectly spherical discs, varying in size up to 100 mμ, are often seen both in the membrane outside of the specimen and in the specimen itself. Secondly, one finds areas of sudden blurring of the cytoplasmic detail occurring with no apparent distribution within the cell. These may well represent destruction of cellular detail occurring during the polymerization of the specimen. Careful comparison of different polymers and methods will undoubtedly elucidate much of this. Despite this, it seems clear that methods of fixation and sectioning are applicable to many current virus problems.

The sections of the cells infected with herpes notably failed to yield evidence of large amounts of virus within the nucleus. Nor was there any marked early change in the nucleus. Many cells, however, contained distended disentegrating mitochondria and cytoplasm with relatively normal nuclei. This would raise the question as to whether virus is actually preferentially inside the nucleus, and whether the well known intranuclear inclusion of herpes, seen in formalin and Zenker's fixed material, is primarily a reaction to cytoplasmic changes. Solution of this must, however, await a more definite identification of the virus particles in sections. It is also well to remember in this connection that acidophilic intranuclear inclusions have been clearly demonstrated in small pox (19) and chicken pox (20), in each of which there is good evidence of cytoplasmic changes presumably due to the virus. Finally there is no direct evidence of a preferential concentration of herpes virus in the nucleus (21).

The question of the adequacy of osmium fixation might be legitimately raised if superior fixatives were available for study of the nuclei by electron microscopy. Furthermore, we have described changes occurring within the nuclei of cells infected with the virus of fowl pox (10). The sharpness and clarity of these structures indicate that osmium is adequate for at least some of the intranuclear material.

[2] We are greatly indebted to Mr. Mark Gettner for a brief comparative study of sections of the same material sectioned by the two techniques.

SUMMARY

An electron microscope study of thin sections of the chick chorio-allantoic membrane is presented. The cells in this membrane show a detail of structure having approximately ten times the resolution of ordinary microscopy. Their normal appearance is described and the preferability of osmium fixation confirmed.

Epithelial cells infected with the virus of herpes simplex show a swelling and disentegration of the mitochondria and an apparent increase in the cytoplasmic fluid. No particles could be definitely identified as virus although a number of roughly brick-shaped particles were found close to destroyed mitochondria. No specific changes were seen in the nuclei of these cells.

Cells of the chorio-allantoic membrane of both epithelial and mesenchymal origin, when infected with vaccinia and a virus recovered from a child with a varicelliform eruption, show clusters of virus particles varying in size. These particles are intensely osmiophilic and have an apparent internal concentration of dense material. They are often associated with mitochondria.

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UNINFECTED CELL FIXED WITH OSMIC ACID. ×24,000

FIG. 1. Section of epithelial cell. Extended and branched mitochondrium at left. Fine fibrous material in cytoplasm near nucleus.

FIG. 1

UNINFECTED CELLS FIXED WITH OSMIC ACID. ×12,000

FIG. 2. Section of epithelial cell. Edge of nucleus has been shoved off in section.

FIG. 3. Tissue culture preparation of epithelial cell from chorioallantoic membrane. Nucleus to left. Branches of mitochondria to right. In general, cell is too thick to get adequate detail.

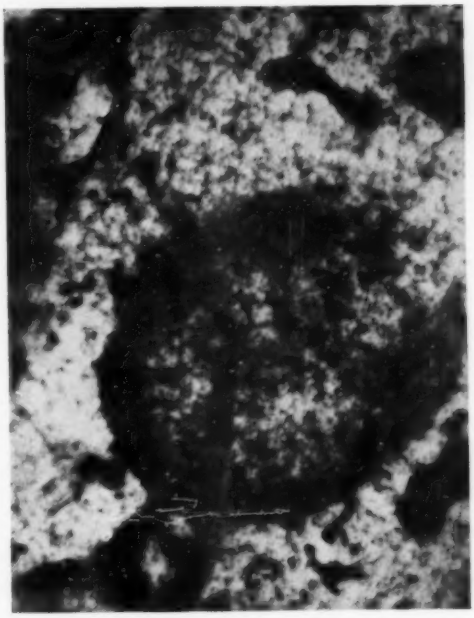

FIG. 2

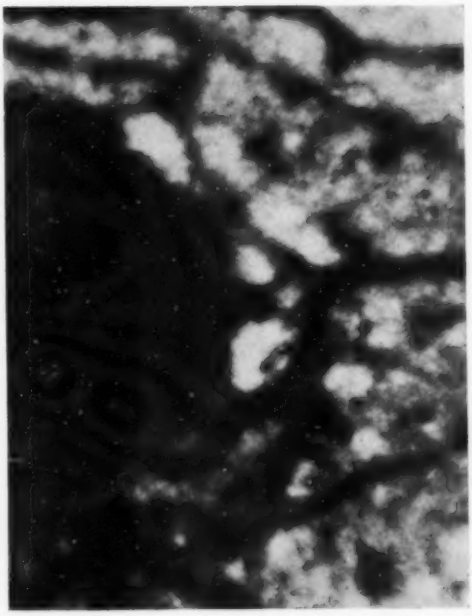

FIG. 3

UNINFECTED CELL FIXED WITH OSMIC ACID. ×24,000

FIG. 4. "Collagen" like material showing variation in periodicity. From subepithelial areas.

FIG 4

SECTIONS FROM CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH HERPES SIMPLEX (H.F.). OSMIC ACID FIXATION. ×24,000

FIG. 5. Cell nucleus to left. Material finely granular. No condensation along nuclear membrane. Beginning fragmentation of mitochondria.

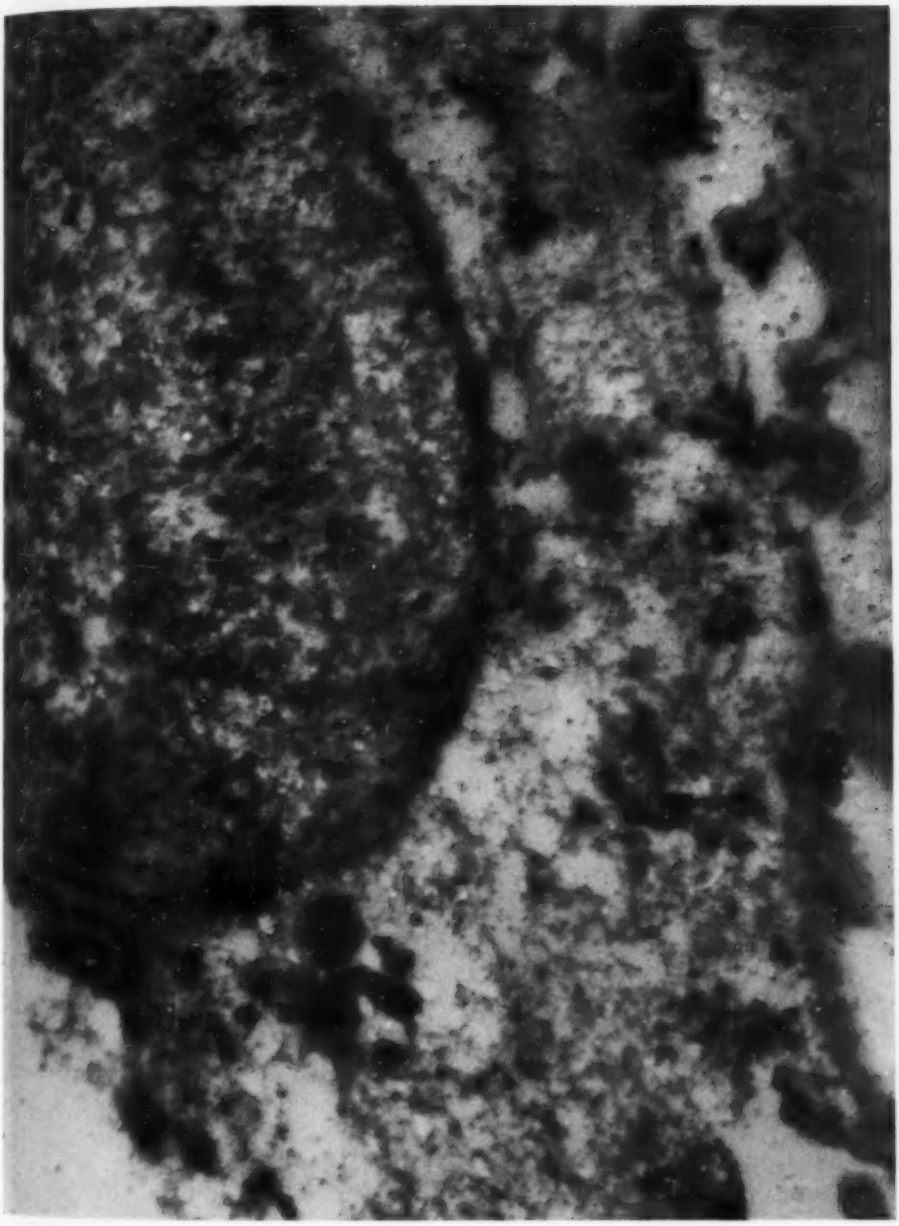

FIG. 5

SECTIONS FROM CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH HERPES SIMPLEX (H.F.) OSMIC ACID FIXATION. ×24,000

FIG. 6. Accumulation of intracytoplasmic fluid. Mitochondria denser. Accumulation of dense particles in cytoplasm.

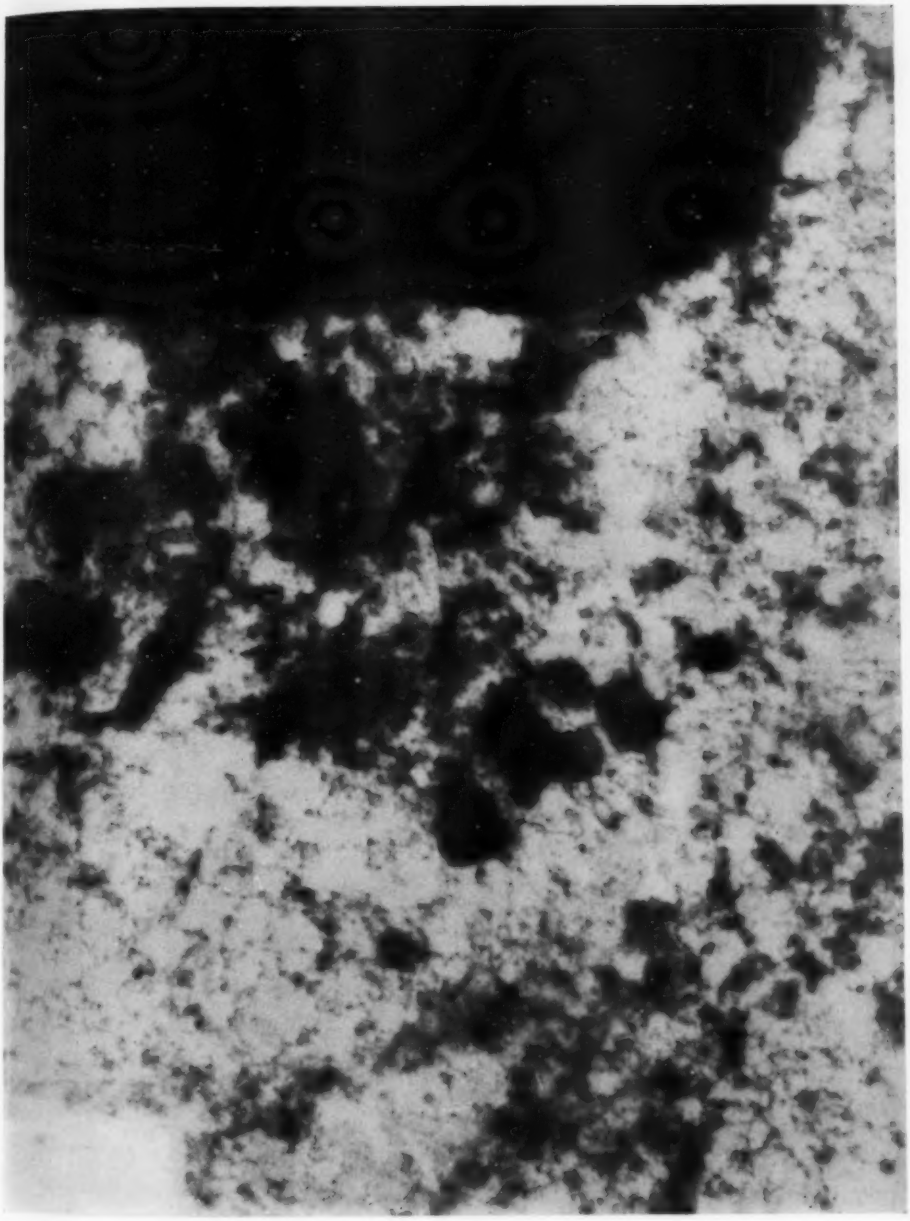

FIG. 6

SECTIONS FROM CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH HERPES SIMPLEX (H.F.) OSMIC ACID FIXATION. ×12,000

FIG. 7. Mitochondria plastered on nuclear surface.

FIG. 8. Variety of dense mitochondria at upper pole of cell. Beginning fragmentation. No apparent changes in nucleus.

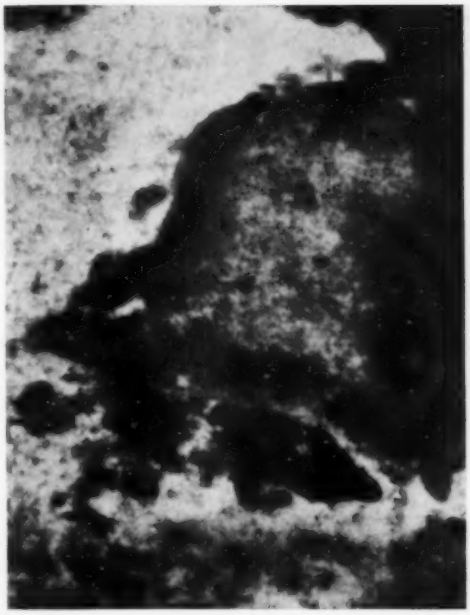

FIG. 7

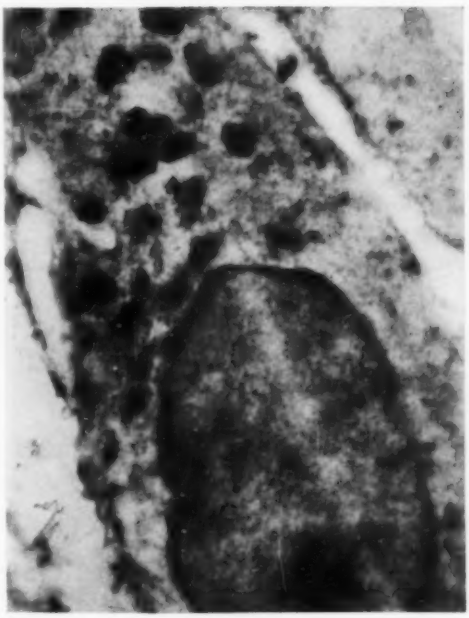

FIG. 8

SECTION OF CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH HERPES SIMPLEX (M.F.). OSMIC ACID FIXATION ×24,000

FIG. 9. Cytoplasmic changes extensive. Mitochondria vesiculated. No apparent nuclear changes.

FIG. 9

SECTION OF CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH HERPES SIMPLEX (M.F.). OSMIC ACID FIXATION ×24,000

FIG. 10. Last stages of mitochondrial disintegration. Dense particles seen near the mitochondria in lower left. Mitochondria show thickening along one side.

FIG. 10

SECTIONS OF CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH HERPES SIMPLEX (M.F.). OSMIC ACID FIXATION ×24,000

FIG. 11. Relatively intact nucleus. Accumulation of finely granular material outside nuclear membrane.

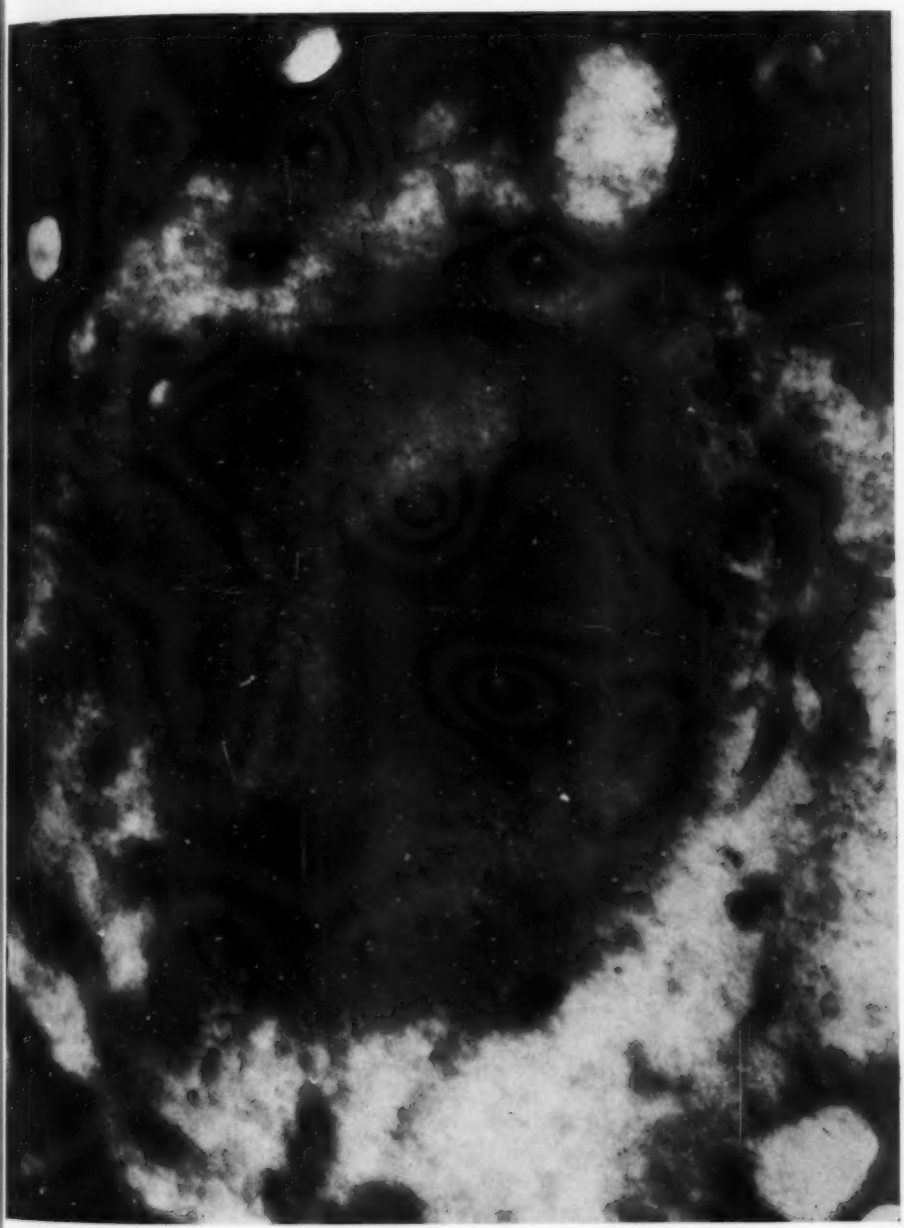

FIG. 11

SECTION OF CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH HERPES SIMPLEX (M.F.). OSMIC ACID FIXATION ×24,000

FIG. 12. Accumulated vesicles, some of which may be mitochondria. In mesenchyme with "collagen" surrounding.

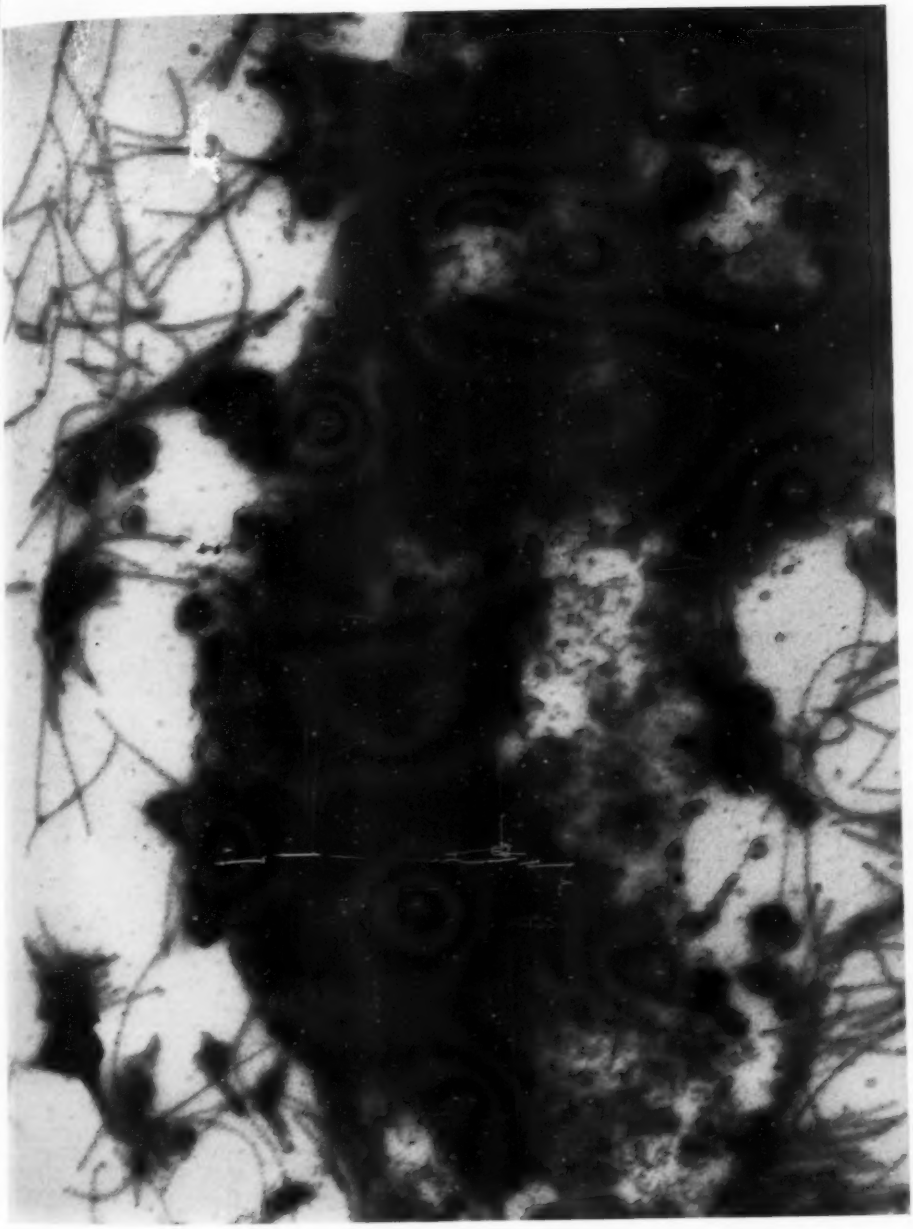

FIG. 12

SECTION OF CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH A VIRUS OBTAINED FROM CASE OF DISSEMINATED VARICELLIFORM ERUPTION ×24,000

FIG. 13. Section of intact nucleus and nuclear membrane. Masses of virus particles seen pushing in on nuclear membrane.

FIG. 13

SECTION OF CHICK CHORIO-ALLANTOIC MEMBRANE INFECTED WITH A VIRUS OBTAINED FROM CASE OF DISSEMINATED VARICELLIFORM ERUPTION ×24,000

FIG. 14. Section showing edge of nucleus showing virus particles scattered and strung out in lines.

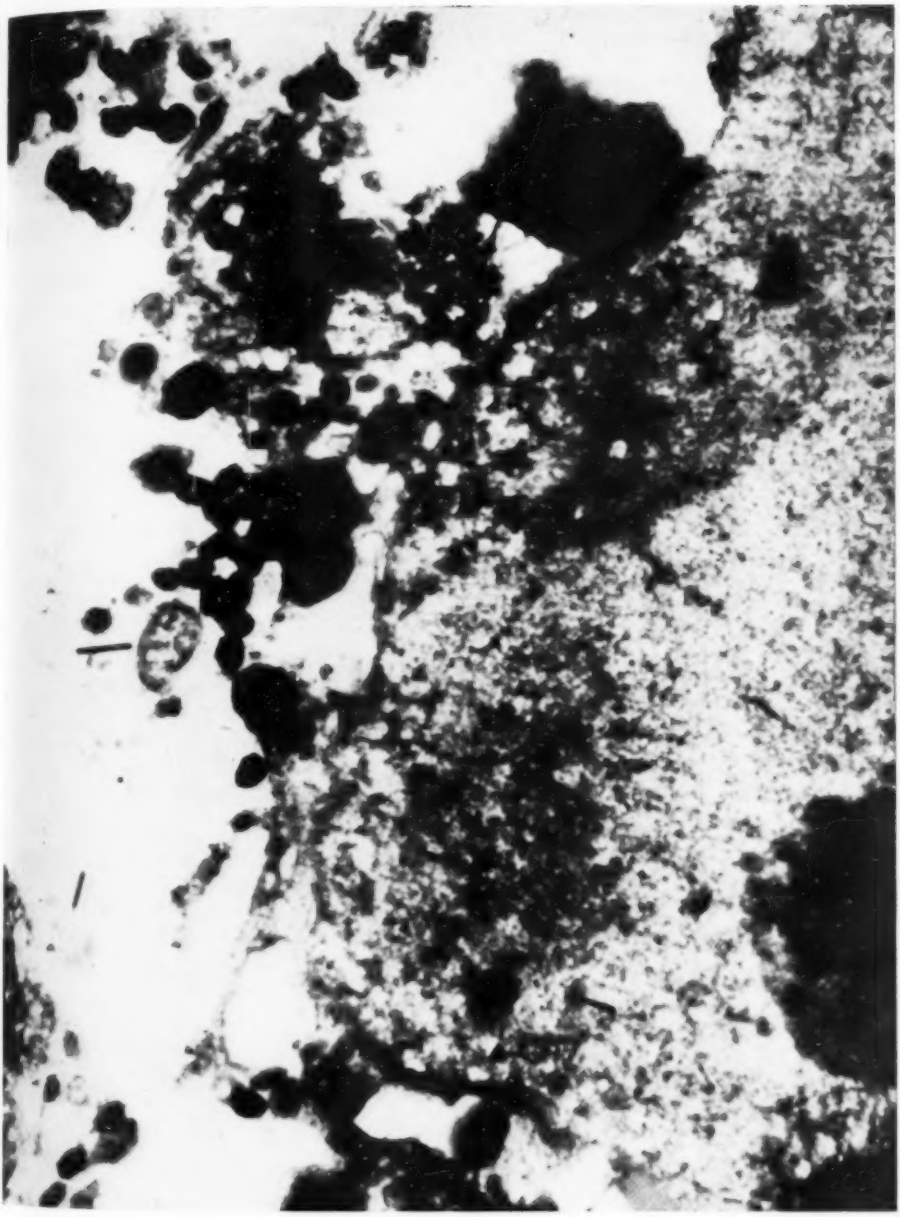

FIG. 14

PLATE IV—*Continued*

SECTIONS AND TISSUE CULTURE OF VIRUS OBTAINED FROM CASE OF DISSEMINATED VARICELLIFORM ERUPTION. OSMIC ACID FIXATION. ×12,000

FIG. 15. Section of portion of cell with distended mitochondrium. Brick shaped particle within has appearance like virus particles.

FIG. 16. Tissue culture preparation. Shadowed with chromium. Mitochondrium in center of field with two brick shaped particles apparently within the cell.

FIG. 15

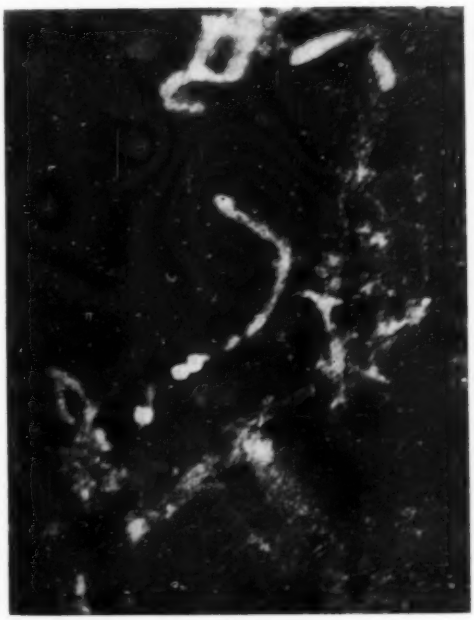

FIG. 16

SECTION OF CHORIO-ALLANTOIC MEMBRANE INFECTED TWO DAYS PREVIOUSLY WITH COMMERCIAL STRAIN OF VACCINE VIRUS ×24,000

FIG. 17

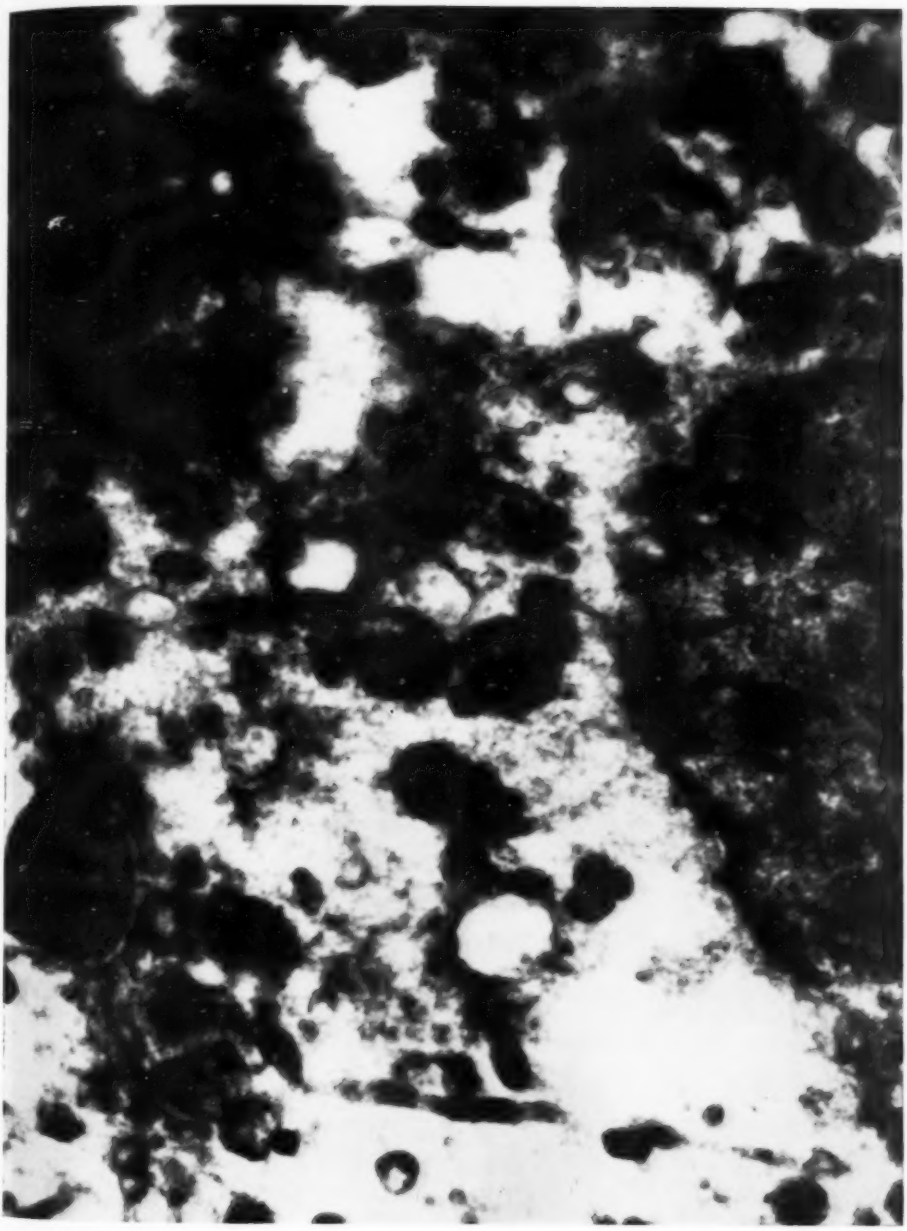

FIG. 17

THE EFFECT OF CORTISONE UPON THE EXPERIMENTAL CARDIOVASCULAR AND RENAL LESIONS PRODUCED BY ANAPHYLACTIC HYPERSENSITIVITY

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In a recent study in this laboratory (1) it was found that animals treated with the adrenocorticotropic hormone of the pituitary (ACTH) during the establishment of hypersensitivity of the serum-sickness type exhibited, in comparison with untreated sensitized controls, a decidedly lessened tendency to develop the cardiovascular lesions (periarteritis nodosa and rheumatic-type lesions) that have been shown to result from protracted anaphylactic reactions (2, 3). In that study, 90 per cent of 20 untreated, sensitized rabbits developed vascular or cardiac lesions or both, whereas such lesions were found in only 25 per cent of 20 sensitized rabbits that were treated with ACTH. While this difference was impressive, it was believed that because of the unpredictable variations in the tendency of individual rabbits to develop lesions of this type as a result of sensitization to foreign protein further studies should be carried out before drawing definite conclusions regarding the inhibitory effect of ACTH upon the development of the lesions. An additional and confirmatory experiment with ACTH has since been carried out (4). The present report will describe the results of a third study in which cortisone, instead of ACTH, was used.

EXPERIMENTAL PROCEDURE

Each of 40 male albino rabbits, averaging about 2700 grams in weight (Table I), was given intravenously a single injection of 10 cc of sterile horse serum (without preservative)[1] per kilogram of body weight. This procedure was used by Rich and Gregory (2, 3) in their studies on the experimental production of periarteritis nodosa, rheumatic-like cardiovascular lesions and glomerulonephritis, and has since been used effectively by numerous other investigators.

Two days before the intravenous injection of horse serum, each animal, under

[1] We are indebted to Sharp and Dohme, Inc. for generously supplying the horse serum.

ether anesthesia, was given an injection of horse serum into the substance of the cornea of each eye. With a 30 gauge, short bevel needle an amount of horse serum sufficient to produce a circular opacity about 5 mm. in diameter was injected into the cornea overlying the pupil. Four normal rabbits, which received no subsequent intravenous injection of horse serum, were injected intracorneally in the same manner.

TABLE I

Ratio of Adrenal Weight to Body Weight at Autopsy

CORTISONE TREATED				SALINE TREATED CONTROLS			
Rabbit	Body weight at autopsy	Weight of adrenals	Adrenal wt. / Body wt. × 1000	Rabbit	Body weight at autopsy	Weight of adrenals	Adrenal wt. / Body wt. × 1000
	Gm	*Mgm*			*Gm*	*Mgm*	
41	2725	177	.065	61	3300	333	.101
42	2450	135	.055	62	3250	294	.090
43	2475	149	.060	63	3050	255	.084
44	2300	244	.160	64	2850	276	.097
45	2875	177	.061	65	2625	269	.102
46	2800	328	.117	66	3000	229	.076
47	2350	192	.085	67	3200	307	.096
48	3375	130	.038	68	2525	230	.091
49	2525	176	.067	69	2825	257	.091
50	2700	197	.073	70	2825	299	.106
51	3500	152	.043	71	2800	243	.087
52	2200	208	.094	72	2450	223	.091
53	2500	198	.079	73	3375	426	.126
54	3125	228	.073	74	2750	213	.077
55	3000	230	.077	75	2500	368	.147
56	2700	375	.139	76	3125	269	.086
57	3100	235	.076	77	3000	321	.107
58	2625	201	.076	78	2425	327	.135
59	2700	148	.055	79	2550	293	.115
60	2225	180	.081	80	3225	261	.081
Averages.....	2712.5	203	.075		2882.5	284.6	.100

Beginning one day before the intravenous injection of horse serum, each of 20 of the 40 rabbits was given daily throughout the experiment a single intramuscular injection of 7.5 mgm of cortisone (0.3 cc of Merck's Saline Suspension of Cortisone Acetate, containing 25 mgm per cc. Two lots of this product were used, #9R6334 and #9R6676).[2] The 20 remaining animals received daily control injections of 0.3 cc of physiological saline intramuscularly.

[2] The cost of the cortisone was defrayed by the U. S. Public Health Service.

Throughout the experiment the animals were kept in separate cages in the same room, and were given an unrestricted amount of Purina Rabbit Chow Checkers and water. No supplement of fresh vegetables was given. Each animal was weighed daily.

The urine of every animal was tested for sugar (Benedict's qualitative test) on at least two occasions during the experimental period. In the control group, except for occasional traces of reducing substance, the tests were consistently negative. All of the cortisone-treated animals showed glycosuria. There was a tendency for the amount of the reducing substance to become somewhat less toward the end of the experiment. A test for acetone was performed whenever the urine showed reducing substance. Acetonuria was never demonstrable.

The rectal temperatures of all animals were taken daily for several days before the beginning of the experiment and throughout the experimental period.

On the thirteenth day after the intravenous injection of serum, all of the animals were tested for hypersensitivity by the intradermal injection of 0.1 cc of undiluted horse serum and 0.1 cc of a 1:50 dilution of horse serum in physiological saline. In addition, 14 of the cortisone-treated and 14 of the control animals were tested at the same time with 1:20 and 1:100 dilutions of horse serum.

On the fifteenth day after the intravenous injection of serum, all animals were killed after taking blood from the heart for serological studies. All serological tests were performed on the sera obtained at this time. The sera from the animals given cortisone, in contrast to those of the control group, were uniformly turbid due to the presence of large quantities of lipide. In order to perform satisfactory precipitin tests, it was found necessary to use some method for clearing the sera. Centrifugalization at 15,000 rpm in the cold for several hours was ineffective. The sera of both the cortisone-treated and the control animals were therefore extracted with ether at −25°C, according to the method used by McFarlane (5) in the study of hemagglutinins. McFarlane found "little or no reduction in agglutinin titre" in blood-grouping sera cleared by this method, but in the present experiments parallel tests on extracted and unextracted non-lipemic rabbit serum containing precipitins against horse serum showed that the extraction procedure reduced the precipitin titre appreciably. The precipitin titres given in Table III, therefore, can be regarded as expressing only relative differences in titre in the sera, all of which were treated in the same manner. Ring tests for the presence of antibody were performed by layering serial dilutions of horse serum over the undiluted serum to be tested, in tubes 3 mm in diameter. The sera of 10 cortisone-treated and of 10 control animals were tested for circulating antigen by layering serial dilutions of the serum to be tested over an antiserum sufficiently potent to give a heavy ring with a 1:25,600 dilution of horse serum. This antiserum was obtained from a rabbit which had been given 1 cc of horse serum intravenously every other day for three injections, and was bled three weeks after the last injection. In these tests for circulating antigen it was unnecessary to extract the test sera to remove the lipides, for the serial dilutions cleared the sera of the cortisone-treated animals sufficiently.

Careful autopsies were performed on all animals. Sections for the microscopic study summarized in Table III were made from the heart, lung, liver, spleen, adrenals, both kidneys, pancreas, testis, skin-test site and eyes of each animal. Other tissues were preserved for additional study. An equal number of sections of the organs of the treated and the control animals were prepared, and thoroughly searched for lesions. The sections of the corresponding organs of all animals were of approximately the same size.

RESULTS

Comparison of the *weights* of the animals at the end of the experiment with those at the beginning showed that 9 of the cortisone-treated animals gained from 50 to 150 grams; 8 lost from 50 to 350 grams (one of these died on the 12th day after the intravenous injection of serum, and showed at autopsy a severe hemorrhagic glomerulonephritis of the type to be described below); and 3 weighed approximately the same as at the beginning. 14 of the control animals gained from 100 to 500 grams; 5 lost from 50 to 250 grams; and one weighed approximately the same as at the beginning.

While there was no consistent pattern of *temperature response*, all treated and control animals had a temperature above 103.5°F. at some period from the fifth day after the intravenous injection of the serum, and in the great majority the temperature reached levels above 104°F. There was no evidence that the cortisone treatment affected appreciably the temperature level. Five animals in the treated group and five in the control group developed temperatures of 105° or above after the fifth day following the serum injection.

The results of the *skin tests* are presented in Table II. It can be seen that the treatment with cortisone did not prevent the development of hypersensitivity, nor did it abolish or markedly inhibit the capacity of the skin of the sensitized animals to react to the injection of the antigen. There was a tendency for the skin reactions to be somewhat smaller in the treated animals than in the controls, and this was the case in our previous study in which sensitized animals were treated with ACTH (1). However, neither cortisone nor ACTH exerted any very dramatic effect upon the skin reactions as observed macroscopically, and there were no significant differences in the microscopic character of the lesions in the treated animals as compared with those in the untreated ones. Table II lists the reactions to un-

diluted serum and to the 1:50 dilution. No greater differences were observed in the case of the 1:20 dilution, and the reactions produced by the 1:100 dilution in the untreated controls were too slight to provide any valid comparison with those in the cortisone-treated animals. The several reactions indicated in the Table as having been unsuitable for satisfactory reading were tests in which the injections had to be made into one of the abnormally thick skin sites that are not uncommon in rabbits. Since four skin tests were made on the flanks at the same time, it was not always possible to find four widely enough separated, normally thin skin sites.

The failure to demonstrate any striking difference between the skin reactions in the treated and control animals does not necessarily mean that cortisone or ACTH cannot effectively inhibit anaphylactic inflammatory reactions which are initiated in ways other than the direct injection of the antigen into the tissues. In most naturally occurring anaphylactic reactions much smaller amounts of antigen reach the site either by way of the blood stream or by absorption from permeable surfaces; and under proper conditions and amount of local antigen deposition, of antibody concentration, and of cortisone or ACTH dosage, anaphylactic inflammation may be inhibited to a much greater degree than in the present skin tests, or may even be entirely suppressed. This, indeed, is evident from the suppression of the anaphylactic inflammatory-necrotizing cardiovascular lesions in the present experiments, and in those in which ACTH was used (1, 4).

The results of the *intracorneal test injections* were interesting. It is well known that the absence of grossly observable erythema and edema at the site of a skin test does not mean that no anaphylactic inflammation has occurred at the site. The amount of inflammation revealed by the microscope at the site of a "negative" skin test is often surprising to those who examine such sites microscopically for the first time. In the present experiments, therefore, an attempt was made to study the effect of cortisone in inhibiting the anaphylactic inflammatory response in a tissue in which lesser degrees of inflammation are more readily observable in the intact animal than is possible in the case of the skin. For this purpose the cornea was chosen, for, though the cornea itself contains no blood vessels, it becomes visibly clouded when it becomes permeated by exudate from vessels at the

TABLE II

Local Reactions 24 Hours after Intracutaneous Injection of 0.1 cc of Horse Serum on 12th Day after Intravenous Sensitizing Injection (Measurements in mm)

CORTISONE TREATED			SALINE TREATED CONTROLS		
Rabbit	Undiluted horse serum	1:50 horse serum	Rabbit	Undiluted horse serum	1:50 horse serum
41	25 × 25 pink edema, 10 × 10 purple center	10 × 15 pink edema	61	30 × 30 pink edema, 10 × 10 purple center	15 × 15 pink edema
42	25 × 30 pink edema, small purple center	8 × 10 pink edema	62	35 × 40 pink edema, 5 × 10 purple center	15 × 15 pale edema
43	20 × 25 pink edema, small red center	10 × 15 pink edema	63	35 × 40 purple edema, 5 × 5 necrosis	15 × 20 pink edema
44	*	*	64	35 × 40 pink edema, 10 × 10 red center	20 × 20 pink edema, small red center
45	20 × 25 pink edema, small purple center	15 × 17 pink edema	65	40 × 60 pale edema, small red center	25 × 30 pale edema
46	25 × 30 pink edema, small purple center	10 × 15 pink edema	66	25 × 35 pink edema	20 × 20 pale edema
47	25 × 40 pale edema, small red center	8 × 10 pink edema	67	35 × 45 pink edema, small purple center	slight pink edema
48	35 × 45 pink edema	15 × 15 pink edema	68	30 × 50 pink edema, 7 × 9 red center	18 × 25 pink edema
49	45 × 50 pink edema, 10 × 10 red center	10 × 15 pink edema	69	30 × 35 pink edema, 5 × 5 purple center	†
50	20 × 25 pink edema	10 × 10 pink edema	70	40 × 45 pink edema, 6 × 6 purple center	†
51	30 × 45 pink edema, small purple center	slight pale edema	71	35 × 40 pink edema, 10 × 10 purple center	10 × 15 pink edema
52	20 × 20 pink edema	0	72	25 × 30 pink edema, small red center	slight pale edema

53	25 × 25 pink edema	10 × 10 pink edema	73	25 × 30 pink edema, 10 × 10 red center	slight pale edema
54	20 × 30 pink edema, small purple center	slight pale edema	74	25 × 30 pink edema, small red center	slight pink edema
55	25 × 25 pink edema	12 × 12 pale edema	75	25 × 35 pink edema, 6 × 6 purple center	10 × 15 pink edema, small red center
56	15 × 20 pink edema	slight pale edema	76	30 × 40 pink edema, 10 × 10 purple center	†
57	40 × 50 pink edema, 10 × 10 purple center	†	77	35 × 40 pink edema, 10 × 10 purple center	†
58	25 × 30 pink edema, small purple center	slight pink edema	78	25 × 35 pink edema, small red center	slight pink edema
59	30 × 40 pink edema, small red center	slight pink edema	79	15 × 20 pink edema	slight pale edema
60	30 × 40 pink edema	slight pale edema	80	30 × 40 pink edema, small red center	15 × 20 pale edema
	Average; 27 × 33 mm	11 average 10 × 14 mm 7 less than 10 mm 1 illegible† 1 untested*		Average; 30 × 38 mm	10 average 16 × 20 mm 6 less than 10 mm 4 illegible†

* Died on 12th day after intravenous injection of serum, before skin test.

† Reaction, if any, illegible because of abnormal thickness of skin site (see text).

limbus. It was shown years ago by Wessely (6) and others that if a small amount of foreign protein is injected into the cornea of a normal animal, the bleb will be rapidly absorbed and the cornea will remain clear for about 9 to 14 days, at which time the cornea becomes cloudy. This clouding results from the fact that the injection sensitizes the animal, and when sufficient antibody has been produced its interaction with any locally remaining, unabsorbed antigen induces an anaphylactic inflammation. It was our purpose to determine whether this readily observable anaphylactic inflammatory reaction would be inhibited by cortisone. All cortisone-treated and control animals were therefore given an intracorneal injection of horse serum at the beginning of the experiment, as described under "Experimental Procedure", and the eyes were observed for the development of corneal clouding. In view of our past experience with the constancy of this reaction, it was surprising that the eyes not only of the cortisone-treated animals but of the controls as well remained perfectly normal. Corneal clouding, however, occurred in all of the four normal animals that were injected intracorneally in the same manner but received no intravenous injection of horse serum. It was evident that the intravenous injection of serum before the development of hypersensitivity had acted to inhibit the flare-up of anaphylactic inflammation in the limbus that occurs between one and two weeks following a primary intracorneal injection of foreign protein. We have repeated and confirmed this observation in a further study of the phenomenon to be reported later. While this unexpected result of the intravenous injection of serum eliminated the opportunity to study the effect of cortisone upon the corneal flare-up reaction, it is, in itself, an observation of no little interest in regard to antigen-antibody relationships in anaphylactic reactions. The positive skin tests and the occurrence of anaphylactic cardiovascular and renal lesions in the same animals in which the corneal reaction was suppressed demonstrates that there was no generalized desensitization. It is evident, therefore, that the occurrence of a local hypersensitive reaction at a given site depends upon proper local conditions and proper antigen-antibody relationships. The effect of cortisone upon the corneal flare-up reaction in animals not subjected to an intravenous injection of serum is at present under investigation.

The results of *precipitin tests for circulating antibody and antigen* are presented in Table III. As was stated under "Experimental Procedures", the presence of *an excessive amount of lipide in the sera* of the cortisone-treated animals necessitated the subjection of the sera to an extraction procedure which lowered the antibody titre. For the purpose of control, this procedure was also applied to the sera of the animals that did not receive cortisone. While the titres recorded in Table III are therefore lower than the actual titres of the sera, they indicate that there is a slight tendency of the titres of the cortisone-treated animals to be lower than those of the control animals, and also that there is no parallelism between the antibody titre and the development of anaphylactic visceral lesions. This was the case in our previous study in which ACTH, instead of cortisone, was used (1). Table III also shows that there was no parallelism between the amount of antigen remaining in the circulation and the development of anaphylactic visceral lesions. The cortisone-induced lipemia is under further study.

The *cardiovascular lesions* of hypersensitivity (periarteritis nodosa, rheumatic-like cardiac lesions) that developed in the animals of this study were altogether like those fully described and illustrated in previous reports from this laboratory (2, 3), and need not be redescribed here. Table III shows that, apart from occasional foci of slight sub-intimal infiltration of mononuclear cells, 17 (85%) of the 20 control animals developed well-marked vascular or cardiac lesions or both, whereas such lesions were found in only 4 (20%) of the 20 cortisone-treated animals. These figures are closely similar to those in our studies in which ACTH was used. In the first of those studies (1), 18 (90%) of 20 untreated sensitized animals developed periarteritis nodosa, endocarditis or both, whereas such lesions were present in only 5 (25%) of 20 sensitized animals treated with ACTH. In a subsequent study of the same type (4), the lesions were present in 16 (84%) of 19 untreated sensitized animals and in only 5 (26%) of 19 ACTH-treated sensitized animals. The results of these three separate experiments, comprising a total of 118 animals all sensitized with horse serum in the same manner and half of them treated with either ACTH or cortisone, provide clear evidence that both ACTH and cortisone exert a decidedly inhibitory effect upon the development of the cardio-

TABLE III

CORTISONE TREATED						CONTROLS					
Rabbit	Antibody titre*	Antigen titre	Periarteritis nodosa	Endocarditis	Slight subendothelial infiltration	Rabbit	Antibody titre*	Antigen titre	Periarteritis nodosa	Endocarditis	Slight subendothelial infiltration
41	1:1600	Not tested	0	+	0	61	1:800	Not tested	+	+	+
42	1:50	Not tested	0	0	0	62	1:400	Not tested	+	+	+
43	1:200	Not tested	0	0	0	63	1:1600	1:200	0	0	+
44†	Not tested	Not tested	+	+	+	64	1:6400	1:10	+	+	+
45	1:1600	0	0	0	+	65	1:400	1:400	+	0	+
46	1:200	1:200	0	0	0	66	1:800	1:10	+	0	0
47	1:800	Not tested	0	0	0	67	1:1600	1:1	+	0	0
48	1:1600	Not tested	0	+	+	68	1:400	1:800	+	0	+
49	1:800	1:200	0	0	0	69	1:400	1:400	+	0	0
50	1:400	Not tested	0	0	0	70	1:800	Not tested	+	0	0
51	1:400	1:200	0	0	0	71	1:800	Not tested	0	+	+
52	1:400	Not tested	0	0	0	72	1:200	Not tested	+	+	+
53	1:800	Not tested	+	0	+	73	1:1600	Not tested	+	+	+
54	1:1600	1:200	0	0	0	74	1:1600	1:100	0	0	0
55	1:400	1:100	0	0	0	75	1:800	Not tested	+	0	0
56	1:400	1:10	0	0	0	76	1:200	1:200	+	0	+
57	1:800	1:100	0	0	0	77	1:800	Not tested	+	0	0
58	1:200	1:100	0	0	0	78	1:3200	1:10	0	0	0
59	1:100	1:10	0	0	0	79	1:400	Not tested	0	+	0
60	1:400	Not tested	0	0	0	80	1:800	Not tested	+	0	0
Total.....			2(10%)	3(15%)	4(20%)				15(75%)	7(35%)	10(50%)

* Antibody titres reduced by extraction of lipids from serum (see "Experimental Procedures").

† Found dead on 12th day after injection of horse serum.

vascular lesions that result from hypersensitivity (Table IV). Considering the well-known differences in individual susceptibility to the development of periarteritis nodosa and other types of lesions caused by hypersensitivity in both the human being and the experimental animal, the correspondance between the percentage of animals that developed the lesions in each of these three experiments was exceptionally good. Why ACTH and cortisone fail to prevent the development of the lesions in some of the treated animals is not understood. It is quite clear from the study of the tissues, however, that

TABLE IV

Incidence of Cardiovascular Lesions in Treated and Untreated Sensitized Animals

	UNTREATED				TREATED			
	Number of Animals	Periarteritis Nodosa	Endocarditis	Either or both	Number of Animals	Periarteritis Nodosa	Endocarditis	Either or both
Experiment I* (ACTH)	20	14	10	18	20	5	2	5
Experiment II† (ACTH)	19	16	4	16	19	5	2	5
Experiment III‡ (Cortisone)	20	15	7	17	20	2	3	4
Total	59	45 (76%)	21 (35.5%)	51 (86.4%)	59	12 (20%)	7 (11.8%)	14 (23.6%)

* See Reference 1.

† See Reference 4.

‡ Present study.

there are marked individual differences in the reactions of the animals to other effects of these hormones, e.g., the degree of glycogen deposition in the liver and of loss of lipide from the adrenal cortex. Of course, the fundamental problem is the mechanism by means of which cortisone suppresses the development of the cardiovascular lesions.

The effect of cortisone upon the *glomerulonephritis* produced by antigen in the sensitized body is a matter of particular interest. In 1943, Rich and Gregory (2) reported the occurrence of acute glomerulonephritis in rabbits subjected to a protracted antigen-antibody contact by the same procedure as that employed in the present experiment, i.e., a single intravenous injection of an amount of foreign

† Found dead on 12th day after injection of horse serum.

protein sufficient to ensure the presence of a residue of the protein in the circulation after the development of antibody. We have since repeatedly observed the development of glomerulonephritis in animals so treated, and the observation has been confirmed by others (8, 9). This glomerulonephritis reproduces with remarkable fidelity the histological appearance characteristic of the many cases of human acute glomerulonephritis in which the striking alteration is a marked increase in the cellularity of the glomerular tuft and a rather bloodless appearance of the tuft. There has been a great deal of discussion regarding the nature of the cells responsible for this striking tuft cellularity in human acute glomerulonephritis, i.e., whether they represent a proliferation of the tuft epithelium, or of the capillary endothelium, or whether they are mononuclear cells derived from the blood. The precise nature of many of the individual cells is peculiarly difficult to establish. In our original encounter with this experimental glomerulonephritis (2) we were particularly impressed by the epithelial swelling and proliferation. Our subsequent studies have impressed upon us the importance of swelling and proliferation of the endothelial cells as well.

In the present study this increased glomerular cellularity occurred in 10 of the 20 sensitized, untreated animals. It was marked in 4 animals, moderate in 4 and slight in 2. In striking contrast, in the sensitized animals treated with cortisone the glomerular proliferation was entirely suppressed, and the tufts were no more cellular than those of normal animals. However, in 13 of the 20 sensitized animals treated with cortisone there developed striking glomerular lesions, characterized by dilatations of glomerular capillary loops, focal necrosis of cells of loops, and the formation of large hyaline masses in the tufts, recalling the focal hyaline masses in the tufts in diabetics and in some cases of disseminated lupus erythematosus. In association with these lesions there was ordinarily much more hemorrhage in the renal tubules than in the proliferative type of glomerulonephritis produced by sensitization alone. These lesions, which we have not encountered in the nephritis of sensitized, untreated animals, but which have their precise counterpart in some cases of human acute hemorrhagic glomerulonephritis, will be adequately described and illustrated in a separate report, where evidence will be presented that in human acute nephritis, also, there tends to be an inverse relation-

ship between the degree of glomerular cellular proliferation and the amount of hemorrhage present in the tubules.

It is of interest that in our studies in which animals sensitized in the same manner were treated with ACTH (1, 4), no such lesions were encountered in 40 treated animals. Six of the 40 treated animals developed slight to moderate glomerular proliferation, but the glomeruli in the kidneys of the remaining 34 were normal. Of the 40 sensitized, untreated controls in those experiments, 22 developed proliferative glomerulonephritis which was of marked to moderate degree in 11 and slight in 11. In the dosage used, and under the conditions of the experiments, ACTH exerted an inhibitory effect upon the development of glomerular damage, while cortisone, though suppressing glomerular cellular proliferation as did ACTH, induced the development of a quite different type of rather severe glomerular damage, leading to a greater degree of hemorrhage from the glomeruli. Further studies of this highly interesting type of glomerulonephritis in sensitized, cortisone-treated animals are in progress. The present observations raise a question as to the advisability of treating acute glomerulonephritis in man with cortisone. ACTH, on the other hand, appears to exert a decidedly beneficial effect in inhibiting the occurrence of glomerular lesions under conditions otherwise favorable to the development of acute glomerulonephritis due to hypersensitivity.

The *livers* of 15 of the 20 cortisone-treated animals contained increased deposits of glycogen which, in 11 of the animals, reached remarkable proportions (Fig. 2). This is a visible expression of the chemically established action of cortisone in increasing the glycogen content of the liver. Fat droplets were prominent in the liver cells of many of the cortisone-treated animals.

The *spleens* of most of the cortisone-treated animals showed no very striking depletion of lymphoid tissue as compared with those of the controls. It was evident, however, on more careful study, that the peri-Malpighian body lymphoid hyperplasia and the acute splenic tumor type of diffuse lymphoid hyperplasia that have been shown by Rich, Lewis and Wintrobe (7) to result from the parenteral injection of foreign protein, were definitely less prominent in the treated animals than in the controls.

A quite unexpected finding was the presence of foci of *extramedullary blood formation* in the splenic sinusoids of 18 of the 20 cortisone treated

animals (Fig. 3). This was marked in 9 animals, of more moderate degree in 1, slight in 8 and absent in 2. No such foci were found in the liver or elsewhere. In only 2 of the sensitized controls were rare, slight foci of extramedullary blood formation found in the spleen. The bone marrow was examined in several animals only, and no abnormality was found. The reason for the prominent extramedullary blood formation in many of the cortisone-treated animals is not clear. Since, apart from pernicious anemia and conditions that cause hypoplasia or destruction of the bone marrow, extramedullary blood formation in the spleen is most commonly associated with a reduction in the number of effectively functioning liver cells, it is tempting to try to relate the extramedullary blood formation in these animals to the massive overburdening of the hepatic cells with glycogen (Fig. 2). While the correlation between the amount of glycogen in the liver and the presence and amount of extramedullary blood formation in the spleen is good, it is by no means perfect, and at present one can say only that both effects result from the administration of cortisone under the present experimental conditions. In 20 similarly sensitized rabbits treated with ACTH for two weeks, the dosage averaging about 17 mg per day (1), extramedullary blood formation in the spleen was present in 14 instances. In only 2 was it marked; in 9 moderate; in 3 slight; and in 6, absent. The glycogen deposition in the livers of those animals was not as marked as in many of the cortisone treated animals in the present experiments. In the 20 untreated sensitized controls in that experiment, foci of blood formation were present in the spleen in 8 animals. In 3 they were moderate in degree. in 5, slight.

SUMMARY

Forty rabbits were sensitized with horse serum in the manner known to favor the development of periarteritis nodosa, cardiac inflammatory lesions and acute glomerulonephritis. Half were treated with cortisone, the remainder serving as controls.

Well marked vascular or cardiac lesions or both developed in 17 of the 20 control animals, and in only 4 of the 20 cortisone-treated animals. This conforms with our previous observations on the inhibitory effect of ACTH upon the development of the cardiovascular lesions of hypersensitivity.

Sensitization by the procedure used in these experiments produced acute diffuse glomerulonephritis with the cellular proliferation of the glomerular tufts that is characteristic of most cases of human acute glomerulonephritis. Both cortisone and ACTH inhibited the development of this proliferative glomerular lesion, but whereas effective treatment with ACTH maintained the glomeruli in a normal state, treatment with cortisone induced the development of a quite different type of severe glomerular damage with accentuated hemorrhage, which has its counterpart in some cases of human acute hemorrhagic glomerulonephritis.

In many of the cortisone treated animals there was a massive deposition of glycogen in the liver, fat droplets in the liver cells, lipemia, and conspicuous extra-medullary blood formation in the spleen.

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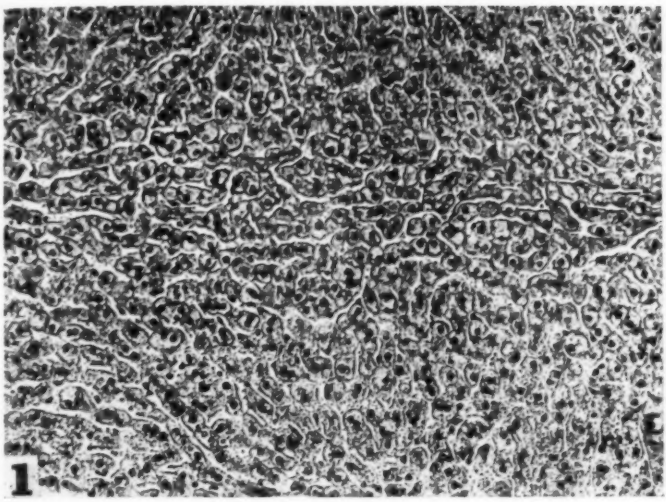
PLATE I

(Photographs by Miss Joan Wheaton)

FIG. 1. Liver of rabbit no. 73, selected to show the greatest amount of glycogen in any rabbit in the control group. Same magnification as Fig. 2.

FIG. 2. Liver of cortisone-treated rabbit no. 50. Note the enormous distension of the cells with glycogen as compared with the control animal in Fig. 1. The cells also contain sudanophilic fat in large and smaller droplets. Same magnification as Fig. 1.



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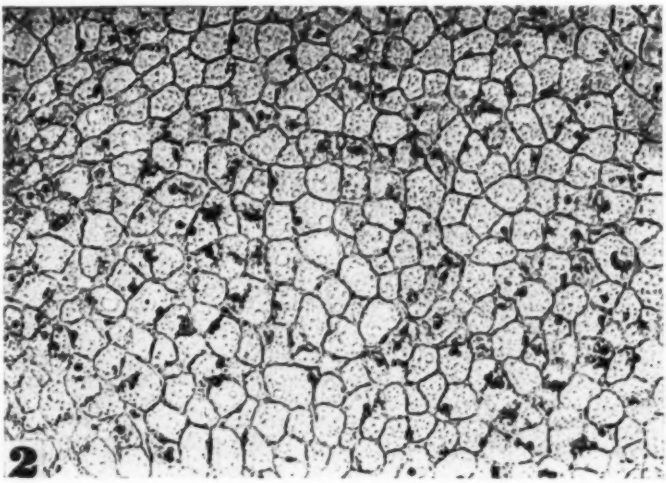

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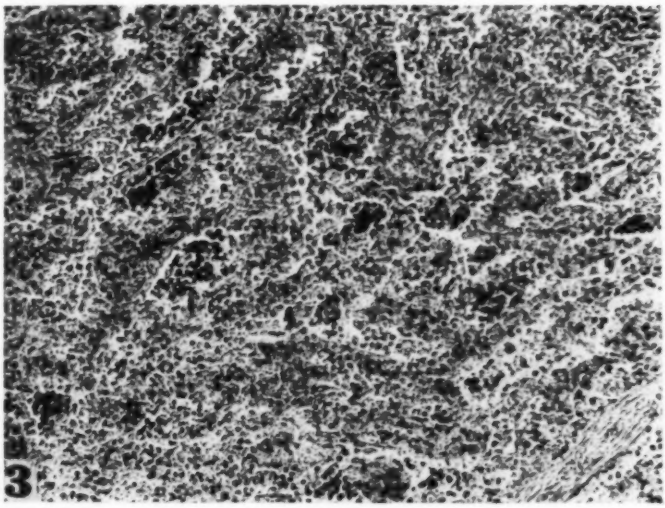
PLATE II

FIG. 3. Spleen of cortisone-treated rabbit no. 48. The black clumps are foci of extramedullary blood formation in the sinusoids.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THE PREVALENCE OF SCURVY AT AUTOPSY DURING THE FIRST TWO YEARS OF AGE*

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INTRODUCTION

This study, dealing with the prevalence of scurvy in children coming to autopsy during the first two years of age, is part of a larger analysis of the effects of specific and nonspecific factors on skeletal tissues begun in 1926 and terminated in 1942. The present communication whose principal purpose is to indicate the prevalence of scurvy will also attempt to correlate certain data derived from the feeding history with the prevalence of this disease as well as to bring out any other ancillary factors. Our concepts of the pathogenesis of scurvy, together with the pathologic anatomy and x-ray changes, will be taken up in communications to follow.

Scurvy, like rickets, is extremely difficult to diagnose clinically because the classical signs and symptoms of scurvy in infancy manifest themselves only when the disease is far advanced. So, too, skeletal lesions must be marked before they can be unequivocally found by x-ray examination. Our own experience on the recognition of scurvy by x-ray will be presented in a forthcoming communication. Although much has been written in regard to blood plasma and white blood cell concentrations of ascorbic acid, there would appear to be a fairly general agreement that such chemical procedures are without value in assessing the incidence of scurvy. Load tests, such as that developed by Kajdi et al. (1), would seem to be more practical and to offer more promise; unfortunately such procedures do not appear to have gained very wide application. The one and only precise method for the detection of scurvy is by post mortem examination, since in the bones of the growing infant the disease declares itself unequivocally under the microscope.

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Although ascorbic acid deficiency leads to certain physiological and biochemical defects in the susceptible organism, the most dramatic changes are morphological ones. These are characterized by an interference with the formation of certain intercellular materials: collagen, osteoid, dentine. In the bone absolute ascorbic acid deficiency leads to a complete dissociation of chondroblastic and osteoblastic activity. Cells of the epiphyseal and costal cartilages continue to proliferate and mature. Inorganic materials continue to deposit in the intercellular cartilage matrix, unless, of course, rickets is also present. Here, however, the analogy to normal sequences of endochondral bone formation ceases. Osteoid, which would ordinarily become bone at once by the depositioe of calcium, phosphorus, carbonate, etc., is not deposited on the calcified cartilage matrix scaffold nor is this latter material removed. In other words, the osteoblast appears to have lost its power to form osteoid and to destroy calcified cartilage matrix. Scurvy is therefore characterized by the develpment at the cartilage-shaft junction of a framework of calcified cartilage matrix or lattice which is devoid of strengthening bone. Such an area is a weakened one and, as a result of muscle pull, fractures of this lattice occur. It is such fractures which give rise to the classical histological picture of scurvy. As was indicated the above brief resume of the pathogenesis of the osseous changes in scurvy is based on absolute or complete vitamin C deficiency such as might be encountered in the experimental animal. Absolute scorbutus is virtually never seen in our human material. That is, there is always some osteoblastic activity as evidenced by deposits of osteoid even in the absence of any direct therapeutic administration of ascorbic acid; the histological picture is changed accordingly.

We shall present in another place a detailed description of the pathological anatomy of scurvy as it affects the skeleton. It seems necessary, however, to define briefly our criteria at this time. At the beginning of this study we had hoped to be able to develop criteria which would enable us to make a definite diagnosis of ascorbic acid deficiency at the earliest possible time, even before the appearance of telltale fractures. In this, however, we were disappointed; we are still not able to make a positive diagnosis in the absence of fractures. Such fractures, which, of course, are seen only on microscopic examination, appear as spicules of calcified matrix lying at various angles to

the portions of intact lattice which is in continuity with the overlying cartilage (Figures 1–4). Usually collections of an homogenous eosinophilic material are found about the fractures and in the clefts between the fragments. Such exudations are often surrounded or invaded by swarms of mesenchymal cells, adult or potential osteoblasts, which may or may not be laying down osteoid about the fractured area. This pink material and osteoid are impregnated with inorganic materials to varying degrees. Lesions thus range from fresh fractures with very little attempt at healing to those which may be completely healed so that only the fracture pattern remains. It should be stated, and we shall elaborate on this point elsewhere, that fractures are not unequivocal evidence for the diagnosis of scurvy. One may observe such lesions in instances of leukemia, following trauma, in rickets, etc. The characteristic feature of scorbutic fractures is that they occur at different levels in the lattice of calcified matrix; this is because they occur at different times. In addition there are certain other findings: hemorrhage beneath the periosteum and about fractures in the medullary cavity, hemosiderin pigment, disappearance of marrow cells, rarefaction, etc. When we have been confronted by only a lattice which has a distinctly scorbutic appearance in that the spicules are increased and devoid of bone, we have felt it was safer to consider such a case one of questionable scurvy and not include it in the series herein reported. Excessive amounts of calcified matrix material or lattice are, of course, not completely specific for scurvy. One may find a similar picture as a result of growth disturbance, congenital syphilis, etc. We have encountered instances in which out of 3 or 4 sections of different bones only one showed fractures (Figures 6 and 7). The others may have had lattices which were abnormal in appearance and highly suggestive of scurvy. Had only these sections been encountered without the one with the damning evidence of the fractures we would have been in the same quandry as we were with others over which our suspicions have been aroused. Since the present study is being used as the basis of an analysis of the incidence and clinical features of scurvy, we have felt it wiser to include only those cases about which we were absolutely sure. Had more sections been available in some of the doubtful group, more cases might likely have been unearthed.

A further important and most disturbing factor in the histological

recognition of scurvy is the presence or absence of rickets. We have briefly alluded in another place (2) to the difficulties encountered in detecting scurvy when severe rickets is present. We shall not dwell further on the coexistance of scurvy and rickets; this will be discussed in more detail elsewhere. It is merely mentioned at this point because subacute and chronic severe rickets may completely obscure the histological criteria of scurvy.

The material upon which this study is based consists of one or more bones from each of a series of 1303 white and negro children ranging in age from the first day to the end of the second year. The series represents a consecutive group of children coming to autopsy from the Harriet Lane Home of the Johns Hopkins Hospital. One or more ribs, usually the middle group, were examined in virtually every instance. Where abailable the epiphyseal portions of one or more of the long bones were also studied. Methods used in the preparation of the specimens have been detailed elsewhere (3). In order to facilitate analysis of the data, special cards (McBee, Keysort) were prepared; pertinent information was either entered or coded on the card and the appropriate holes punched.

A word might be added concerning the racial distribution of the subjects examined. It will be noted that the negro children (total 757) outnumber the whites (total 546). The ratio of negro:white in the City of Baltimore is about 20:80. It therefore seemed of importance to determine whether the hospital population differed from the population as a whole, whether there were more deaths in the negro or whether the autopsy percentage was higher in the negro than in the white children. Selected years have been examined with these points in mind.[1] The inpatient population of the Harriet Lane Home as gauged by the discharges per year indictes that the ratio of white to negro is the same. This is out of proportion to the population as a whole. The increased number of negros in our material is best explained by the fact that the autopsy percentage is higher in this race than in the white. For instance during a 5 year period 1937–42 the total percentage of white children under 2 years of age which were autopsied was 66.5% while 80.6% of all negro children came to autopsy.

[1] We are indebted to Dr. R. V. Rider of the Statistics Department, Johns Hopkins Hospital, for these data.

THE PREVALENCE OF SCURVY BY AGE

In Table I are presented the total number of cases of scurvy, white and negro, occurring at various ages during the first two years. One case of scurvy was found during the first month at 19 days of age; this case, which has been reported in detail elsewhere (4) was regarded as one of congenital scurvy, the only adequately studied case, as far as we are aware, which has been placed on record. It will be noted that no cases of scurvy were found during the next month. However, during

TABLE I

Prevalence of Scurvy by Age and Color

AGE	NO. SUBJECTS EXAMINED	NO. WITH SCURVY	% WITH SCURVY	NO. WHITE SUBJECTS	NO. WITH SCURVY	NO. NEGRO SUBJECTS	NO. WITH SCURVY
0–15 da.	360	0	0	174	0	186	0
16–30 da.	90	1	—	41	1	49	0
1 mo.	99	0	0	48	0	51	0
2 mo.	90	2	2.2	34	2	56	0
3 mo.	69	3	4.3	31	3	38	0
4 mo.	79	2	2.5	26	1	53	1
5 mo.	73	12	16.5	25	3	48	9
6 mo.	53	13	24.5	19	5	34	8
7 mo.	54	10	18.5	22	7	32	3
8 mo.	40	5	12.5	18	2	22	3
9 mo.	38	10	26.3	12	4	26	6
10 mo.	41	5	12.2	16	0	25	5
11 mo.	40	3	7.5	12	0	28	3
12–23 mo.	177	3	—	68	1	109	2
Total.....	1303	69		546	29	757	40

the 2nd, 3rd and 4th months a few cases began to appear and when the 5th month was reached there was a sudden increase which was sustained for the next 6 months after which time there was just as precipitous a fall, so that during the second year only 3 cases were observed, at 12, 13 and 18 months respectively. No case has been recognized at autopsy after the 18th month.

THE PREVALENCE OF SCURVY BY RACE

In Table I is shown the incidence of scurvy in different racial age groups. If one examines the 7 month period in which most of the cases

of scurvy occur, i.e. the 5th through the 11th month, it is found that of the 124 white children, 22 or 18% had scurvy; of the 215 negro children, 37 or 17% had scurvy. Race would therefore appear to play no role in the development of scurvy. In order to be certain that there were no outstanding differences in feeding practices, the two groups have been analyzed from the standpoint as to whether breast milk alone, breast supplemented with cow's milk, breast followed by cow's milk or cow's milk alone were administered. These data are presented in Table II where it will be noted that there were virtually no differences in feeding habits.

TABLE II

Feeding History of White and Negro Infants from the 5th through the 11th Month

	BREAST EXCLUSIVELY	BREAST-COW'S MILK EXCLUSIVELY	COW'S MILK EXCLUSIVELY	BREAST FOLLOWED BY COW'S MILK	UNKNOWN
White	10.0%	4.5%	37.0%	41.5%	7.0%
Negro	11.5%	6.5%	33.0%	41.0%	8.0%

THE RELATIONSHIP OF PREMATURITY TO THE DEVELOPMENT OF SCURVY

An analysis has been made of the possible relationship of prematurity to the incidence of scurvy. Of 577 children from the second through the eleventh month 90 were premature. Of these 10 (11.1%) had scurvy. Of the 487 full term babies 55 (11.3%) had scurvy. It would thus appear that there is no relationship of prematurity to the prevalence of scurvy.

THE RELATIONSHIP OF THE PREVALENCE OF SCURVY TO THE FEEDING HISTORY

Because of the obvious importance of the type of feeding employed during infancy an analysis has been made of that period, i.e. from the 5th through the 11th month, in which the greatest number (85.5%) of cases occurred. The type of milk offered: breast exclusively, a mixture of breast and cow's milk exclusively, cow's milk exclusively, and breast milk for varying periods followed by cow's milk, as well as the intake of vitamin C have been investigated. These data in the case of the types of milk administered are summarized in Table III.

It will be noted that in the first 2 groups, that is among those infants receiving at least some breast milk all their lives, no cases of scurvy were encountered at autopsy.

This group of 58 cases has been further examined from the standpoint of vitamin C administration in the form of orange juice, tomato juice or synthetic ascorbic acid as to whether the vitamin intake was deemed adequate, inadequate or none at all was received. We have been greatly handicapped in our analysis of the relationship of the vitamin C administration to scurvy because of the unreliability of

TABLE III

Relationship of Type of Feeding to the Incidence of Scurvy from the Fifth through the Eleventh month

AGE	TOTAL NO. SUBJECTS	BREAST ALONE		MIXED		COW'S MILK ALONE		BREAST THEN COW'S MILK		UNKNOWN	
		Tot.	Sc.*	Tot.	Sc.	Tot.	Sc.	Tot.	Sc.	Tot.	Sc.
mo.											
5	73	4	0	5	0	36	11	20	1	8	0
6	53	8	0	5	0	20	9	18	3	2	1
7	54	5	0	3	0	16	6	24	2	6	2
8	40	5	0	4	0	13	3	15	1	3	1
9	38	3	0	1	0	10	4	20	5	4	1
10	41	8	0	0	0	15	3	17	1	1	1
11	40	4	0	3	0	12	2	19	0	2	1
Total.....	339	37	0	21	0	122	38	33	13	26	7

* Sc. Scurvy.

many of our histories. Although some infants in our series were private patients whose histories could be assumed as exact, all in which scurvy was discovered were dispensary patients. In the large portion of the dispensary patients of the Harriet Lane Home the mothers were so unintelligent or unreliable that what was reported could not be accepted on its face value and in some cases the infant was brought in by some member of the family other than the mother or by a friend who did not possess the desired information. Moreover in many instances the intern investigating the history did not obtain the accurate detailed information required. This was particularly true in the case of infants so sick that the house officer's duty was to get

them into the ward with the least possible delay. We were able to be absolutely certain in regard to the validity of the vitamin history under two circumstances. (1) When the positive statement was made that vitamin C had never been given and (2) when the child was in the Harriet Lane Home and the vitamin administration was recorded in all its details. Of course, in certain of the dispensary patients the parents were known to be completely reliable and the information in regard to vitamin administration could be accepted. When a specific statement was made that an insufficient quantity of vitamin had been given, namely, that only a teaspoonful of orange juice or a drop of cod liver oil had been given daily or that the vitamin had been given only "occasionally" or "irregularly" or that it had been offered but the infant had refused to take it or had vomited it or when the mother supplied the information that in the entire life of the infant she had bought only 10 oranges or one bottle of cod liver oil we felt that we could be certain that the administration had been insufficient. It is an interesting fact that many mothers will state that orange juice (or vitamin D) have been given in adequate amount regularly when such is not the truth in order to make a good impression, or not make a bad impression, with the examining doctor. When we have been in doubt in regard to the validity of the history of vitamin administration, we have classified the vitamin administration as unknown. In summary, when we have been certain in regard to our information we have used it as being certain and in other instances we have used our discretionary powers, erring on the side of not accepting information as valid when in doubt.

In our estimations of vitamin C administered, we took no account of green vegetables, assuming that the amount of vitamin C supplied by them was too small to be of special importance. Potato was given only in a few exceptional instances.

The results of our analysis of the status of vitamin C administration in the various feeding groups, i.e., breast exclusively, mixed, cow's milk exclusively and breast followed by cow's milk after varying intervals are shown in Tables IV, V and VI. By adequate we have grouped those infants whose history stated that vitamin C, usually in the form of orange juice, was administered during life for an extended period either at home or in the hospital. Cases termed inadequate are

those in which the history states that orange juice was administered "occasionally", "now and then", etc. or was given terminally for only a short period in the hospital. Those in which there was a definite statement of no vitamin administration are grouped under the designation "none".

It is unfortunate that the breast alone-mixed group is so small, only 17% of the total, and particularly disappointing is the paucity of infants definitely said to have received no supplemental vitamin C. In addition, for reasons which will be brought out below it is further disappointing that we do not have any information as to the proportions of breast and cow's milk administered to the mixed feeding group.

TABLE IV

Breast and Mixed Feedings Entire Life in Relation to Vitamin C Intake from Fifth through Eleventh Month

VITAMIN C ADMINISTRATION	BREAST EXCLUSIVELY NUMBER OF CASES		BREAST—COW'S MILK EXCLUSIVELY NUMBER IN CASES	
	With Scurvy	Without Scurvy	With Scurvy	Without Scurvy
Adequate	0	14	0	9
Inadequate or Questionable	0	7	0	4
None	0	14	0	4
Unknown	0	2	0	4

A similar analysis has been made of the infants fed cow's milk and those to whom breast milk was administered for varying periods followed by cow's milk. It will be observed from Table III that there was a higher incidence in the former group than in the latter. Scurvy was found in 32% of the infants fed cow's milk alone while skeletal lesions appeared in only 10% of the breast followed by cow's milk group. The former has been analyzed with regard to vitamin C administration, the results being shown in Table V. It will be noted that over half (59%) of the infants with scurvy received no vitamin C. On the other hand a smaller yet definite proportion (21.7%) of the group showing no scurvy were said to have never received any vitamin C. This group will be discussed further below.

In Table VI is found a similar breakdown for the breast followed by cow's milk group. The cases have been divided into three categories on the basis as to what proportion of their lives they received cow's

milk. All ages have been considered together. The number of cases involved is too few to draw any very definite conclusions, particularly since such a large number (56%) of this group have a history of adequate vitamin C administration.

We have reviewed those cases falling in the age period from the 2nd through the 11th month in whom there is a history of no or excessively

TABLE V

Cases, Aged 5 through 11 Months Receiving Cow's Milk Entire Life in Relation to Vitamin C Administration

VITAMIN C ADMINISTRATION	TOTAL CASES			
	With Scurvy		Without Scurvy	
	Number	Per cent	Number	Per cent
Adequate	5	13.7	57	68.6
Inadequate	6	15.8	2	2.4
None	24	63.2	18	21.7
Unknown	3	7.9	6	7.3

TABLE VI

Cases, Aged 5 Through 11 Months, Receiving Breast Milk for Various Periods Followed by Cow's Milk in Relation to Vitamin C Administration

VITAMIN C ADMINISTRATION	PERCENTAGE AGE BREAST MILK ADMINISTERED					
	25% Scurvy		50% Scurvy		75% Scurvy	
	With	Without	With	Without	With	Without
Adequate	0	33	2	19	0	20
Inadequate	3	12	1	3	0	1
None	2	14	2	1	0	1
Unknown	3	9	0	6	0	1

inadequate vitamin C administration. This was done because our curiosity was aroused by the absence of scurvy in such infants who were receiving cow's milk or breast followed by cow's milk for varying periods. As was pointed out in our introductory remarks rickets may modify or even obscure the morphological evidences of scurvy. Among 78 cases in which there was a history of no vitamin C administration a number of instances (60%) of advanced rickets were encountered.

How much these have masked any scorbutic lesions which may have been present can only be surmised. The reasons for the absence of scurvy in the remaining 40% are even more obscure.

THE RELATIONSHIP OF SEASON TO THE PREVALENCE OF SCURVY

In Table VII have been grouped the percentage incidence of scurvy in children dying in each of the 4 seasons of the year. Although statistically significant differences are present at about the 5% level, we feel that not enough cases are available for study to comment further.

TABLE VII

Prevalence of Scurvy in 577 Autopsies, Age 2 through 11 Months by Season

SEASON	TOTAL CASES	NO. WITH SCURVY	% WITH SCURVY
Winter (Dec.–Feb.)	142	18	12.7
Spring (March–May)	127	9	7.1
Summer (June–Aug.)	119	21	17.7
Fall (Sept.–Nov.)	189	18	9.5

THE SCORBUTIC GROUP

It would seem worthwhile now to turn to a consideration of the entire 69 cases of scurvy, which, as will be recalled from Table I, were found at autopsy as early as the 19th day of age and as late as the 18th month. Adequate data as to feeding were available in 61 of these, 42 of whom were fed cow's milk all of their lives, while 19 received breast milk for varying periods followed by cow's milk. In the cow's milk group there were 32 out of the 42 subjects whose history of vitamin C intake was available. Of these 35 children 23 or 72% had a history of no supplemental vitamin C whatsoever. The remaining cases have been summarized in Table VIII. There were 12 subjects among the 19 who had been fed breast milk followed by cow's milk whose vitamin C intake was known. Of these 6 had a history of no supplemental vitamin C. The remaining cases in this group are summarized in Table IX.

It will be noted that in both these groups varying amounts of ascorbic acid were administered, sometimes adequately, sometimes inadequately. One is struck by the amount of healing which was en-

countered. We mentioned earlier that we did not encounter scurvy in the human without some evidence of healing in contrast to what one may find in the experimental animal dying in absolute scorbutus.

A further important point which is brought out by the above 2 tables is the fact that scurvy has been diagnosed at autopsy despite the fact that certain of these infants had received more than adequate vitamin C. This is because we are able to detect the morphological

TABLE VIII

Cases of Scurvy Fed Cow's Milk and Varying Amounts of Vitamin C

NO.	AGE	VITAMIN C ADMINISTRATION	TYPE AND DURATION ILLNESS	STATUS OF SCURVY AT AUTOPSY
	Mos.			
108	5	15 cc O. Juice last 5 days in hospital	Generalized tbc., Chronic	Healing and healed
161	5	15 cc O. Juice last 13 days in Hospital	Lobular pneumonia, 28 days	Healed fractures
670	5	1 oz. O. Juice last 2 mos.	Lobular pneumonia, Acute	Healing and healed
116	6	15 cc O. Juice last 10 days in hospital	Lobular pneumonia, 20 days	Healing and healed
1721	6	50 mg. ascorbic acid 8 days in hospital	Pneumonia, acute	Fresh. Slight healing
1545	7	2 oz. L. juice every day "recently".	Pneumonia, Acute	Fresh, healing and healed
1352	9	Adequate O. Juice and vitamin C 29 days in hospital	Subdural hemorrhage, Chronic	Healing
1441	11	O. Juice "now and then".	Burns, Acute	Fresh and Healing
1452	12	Large amounts O. juice last month	Burns, Sepsis 1 month	Healed

evidences of ascorbic acid deficiency for some time after the lesions have healed. In Case No. 1410, for instance, the disease had been recognized 2 months before and intensively treated. Yet even after this time telltale marks of preexisting scurvy were present in the cortex of the rib.

Another example is even more illuminating because circumstances were such as to provide us with a most unique controlled study of healing scurvy in the human (Figures 8 and 9) Nos. 1441 and 1452

were twins, 11 months old, at the time of admission. Both were severely burned after upsetting a kerosene stove on themselves. No. 1441 died shortly after reaching the hospital. At autopsy marked scurvy was found. On the basis of this the other child (No. 1452) was intensively treated; the baby survived for 1 month, dying of streptococcal sepsis and meningitis. In this child the scurvy was completely healed yet abundant evidence of its preexistance could be found. As will be noted

TABLE IX

Cases of Scurvy Fed Breast Milk, Followed by Cow's Milk with Varying Amounts of Vitamin C

NO.	AGE	DURATION OF BREAST FEEDING	VITAMIN C ADMINISTRATION	TYPE AND DURATION OF ILLNESS	STATUS OF SCURVY
	Mos.				
981	2	19 days	30 cc O. Juice last 17 days	Pyloric stenosis, Chronic	Fresh and healing lesions
1284	5	2 mos.	100 mg ascorbic acid I.V. 5 and 4 days ante mortem	Dysentery, 28 days	Healed fractiures
244	6	1 mo.	30 cc O. Juice last 8 days in hospital	Dysentery, 1 month	Healing and healed
654	6	21 days	O. Juice 1 tsp. since 4th mo.	Enteritis, Acute	Fresh and healing
1290	9	4 mos.	60 cc. O. Juice last 10 days in hospital	Lobar pneumonia, 16 days	Healing
1410	13	10 mos.	Large amounts Vitamin C 2 mos. before	Tuberculosis Chronic	Healed. Only lesions in cortex remain.

most of the cases which have been classified as scurvy and which have received adequate vitamin C fall into the healing or healed group.

Of the 69 cases of scurvy two thirds (65.4%) were associated with an acute illness. By acute we mean one whose duration was not over 2 weeks. Fewer (20.3%) suffered from illness of intermediate duration (15–30 days) while 14.3% were found in children having chronic disease (1 month or more). Most of the acute illnesses were of infectious origin of various kinds. It is, of course, well known that infections raise the requirements for vitamin C. In this group, however, it would appear that in virtually every instance the scurvy antedated the

terminal infection. Whether there was any relation between the two is, we feel, impossible to determine.

Of the 69 cases of scurvy in our study scurvy was recognized clinically in only 6 instances. The reasons for the failure to recognize scurvy during life can be explained as follows: In some instances undoubtedly the scurvy was not sufficiently advanced to give signs of its presence and could not have been recognized clinically. In other instances scurvy was confounded with rickets and the physical signs of scurvy were interpreted as those of rickets. The recognition of scurvy in the presence of rickets is particularly difficult and, as a later publication will show, the two deficiency diseases occur in combination. In one instance the staff were thrown off the diagnosis of scurvy when the x-ray department reported that the condition was rickets and not scurvy. It was impossible to recognize tenderness of the extremities and pain on motion in the presence of inflammations of the meninges or subdural hematoma or in a desperately sick infant. In a number of instances either the child was brought in dead or the period in the hospital before death occurred was so brief that no satisfactory examination was possible. In a number of instances, also, the study of histories makes it evident that the thought of the clinicians was so concentrated on the obvious, often fulminating, disease from which the child was suffering that the existance of an apparently unrelated deficiency disease did not enter the mind.

The physical sign which was most helpful in the recognition of scurvy proved to be the characteristic ridge enlargement of the costochondral junction. It is noteworthy that hemorrhagic phenomena were present in only two or three instances. The main guide to the existence of scurvy obviously should have been the history of prolonged vitamin C deficiency and not enough weight was given to this.

Elsewhere (11) attention has been called to the association of scurvy and sudden unexplained death, 3 such cases having been reported. We have encountered 2 other such instances in this series so that in all 5 of the 69 cases comprising this study died suddenly and nothing save scurvy was found at autopsy.

DISCUSSION

To our knowledge no study of the autopsy prevalence of scurvy similar to the one herein reported has ever been carried out. Nor

would it appear that material such as this can ever be collected again, in this country at least. The tremendous number of children dying of acute infectious disease in this group of cases has been sharply cut today by chemo-therapeutic agents, so that the whole pattern of children's disease encountered at the autopsy table has changed materially.

Our data on the prevalence of scurvy at autopsy coincide with the scattered clinical observations which have been reported from time to time. Over half a century ago Thomas Barlow (5) stated "The age at which we ought to be on the *qui vive* for the initial symptoms of scurvy is about the eighth month of infant life". In a clinical study reported from the Harriet Lane Home (6) 15 years ago, the peak incidence fell at the 7th, 8th and 9 months. A similar study (7) from the Infants' and Children's Hospital in Boston draws like conclusions as to the age incidence of scurvy.

From the data presented in this study there would appear to be no racial predisposition to scurvy. As was noted above the feeding habits of the white and negro groups appeared to be about the same. The absence of any racial susceptibility is, of course, in contrast to rickets which in Baltimore tends to occur oftener and in a more severe form in the colored child (3, 10).

Our data indicate no relationship of prematurity to the prevalence and severity of scurvy. Here again as is shown elsewhere (10) the role of prematurity on the prevalence of rickets is clearly defined.

The virtually complete absence of scurvy in breast fed infants has been commented on ever since the end of the last century when Barlow described the disease. Barlow (5) himself stated, "In no single case at the time of onset of the malady has the child been breast fed". Our findings, particularly in the breast fed infants who, as the history stated, never received any supplemental vitamin C, are of importance since they may be expected to give certain clues to the vitamin C requirements at various age periods. As is well known human milk contains appreciable amounts of vitamin C, while cow's milk, as a result of processing, contains very little. The vitamin content of human milk varies as to the intake and state of nutrition of the maternal organism. In this country, at least, from an extensive study carried out by Munks et al. (8) the value of 5 mg ascorbic acid per 100 cc of milk may be taken as average. These investigators point out daily

and individual fluctuations so that it must be borne in mind that values as high as 7.0 mg or as low as 3.6 mg per 100 cc may be encountered. Taking 5.0 mg. per 100 cc. as an average value, Clements (9) has calculated the theoretical vitamin C intake at different age periods as follows:

Age in Weeks	4	8	12	16	24
Average Daily Consumption of Breast Milk (oz.)	20.0	25.0	30.0	32.5	35.0
Theoretical Vitamin C Intake (mg)	28	35	43	46	51

From such theoretical considerations it would appear that the ascorbic acid needs of the infant must fall somewhere below the above values. This would seem to be a valid assumption because of the absence of scurvy in the 4 infants fed mixtures of breast and cow's milk and no added vitamin C. This, to be sure, is a small group. Unfortunately, as has been pointed out, we do not know the relative proportions of the two types of milk in these infants' diets.

Another important point which must be considered in the group fed breast milk alone or a breast-cow's milk combination, both types without supplemental vitamin C, is the store of vitamin derived from the maternal organism *in utero*. As is well known, the plasma concentration of the cord blood at delivery is higher than that of maternal blood so that it would appear that the placenta may selectively remove vitamin C from the maternal circulation. How much of this vitamin is stored in the new born is a subject worthy of pointed study because of the bearing it may have on reducing the exogenous requirement for a certain interval after birth. The fact that no cases of scurvy were observed between the first and second months and that the incidence was low during the ensuing 3 months would certainly suggest that the organism can get along ordinarily, for a few months at least, without supplemental vitamin C. In the absence of the vitamin from then on to the end of the first year the presence of scurvy should be seriously considered, particularly in an infant fed cow's milk exclusively. Another factor which must also be taken into consideration in precipitating the sudden increased incidence of scurvy is the tremendous growth activity during this period. This, as is well known, is dramatically shown by the greater incidence of lesions in the most rapidly growing bones. The precipitous drop in prevalence after the first year may be explained by slowing up of growth and, doubtless of greater importance,

by the change in feeding habits because of the addition of potato and vegetables to the diet. The amount of vitamin C so derived doubtless varies considerably depending on methods used in preparation. However, it would appear that at least enough ascorbic acid is ingested to reduce the prevalence of scurvy to practically nothing.

The vitamin C requirement of the premature is usually placed above that of the full term infant. In our series we have only 2 prematures aged 2 and 3 months, who received breast milk exclusively and no supplements of vitamin C. Neither showed scurvy. A third premature, aged 8 months, was on mixed feeding and received no vitamin C. Unfortunately the bones of this subject showed chronic severe rickets which may have masked any scurvy present.

As was noted above healing phenomena were a prominent part of certain infants to whom no supplemental vitamin C had ever been given until the terminal hospital admission which lasted varying periods. From such cases we are able to gain some idea of the healing sequences of scurvy in the human. One must, of course, take into consideration certain variables: approximate ascorbic acid desaturation of the organism, the daily needs, especially in the face of increased metabolism, and infection, theoretical failure of absorbtion as a result of various digestive disturbances, etc. We have seen pronounced healing 8 days after intensive vitamin C therapy was initiated. On the other side of the picture we have found histological evidence of scurvy 2 months after more than adequate therapy. In a case in which there was a 3 months interval between therapy and autopsy no histological evidence remained to indicate the previous presence of scurvy which had been characterized in the x-ray.

Attention should be drawn to the small number of cases (6 out of 69 or 8.7%) in which a definite diagnosis of scurvy was made clinically. This point seems particularly significant to us and would appear to indicate that a number of instances of scurvy may be completely missed in the clinic. If such a small number reveal themselves clinically may not the general prevalence be higher than we are accustomed to expect?

SUMMARY

The prevalence of scurvy during each month of the first year and for the entire second year has been presented for a group of 1303 children studied at autopsy during the period 1926–1942.

There was no racial difference in the prevalence of scurvy.

No cases of scurvy were encountered in children fed breast milk entirely or in part all their lives.

A correlation of the prevalence of scurvy with the vitamin C intake is presented.

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PLATES I AND II

PLATE I

FIG. 1. No. 1299. Low power view of costochondral junction to show characteristic scorbutic fractures, hemorrhage and cellular marrow devoid of myeloid elements. The spicules of fractured cartilage matrix are lying in a disorderly fashion. This was a 9 months old child fed on the breast for 6 weeks and on cow's milk thereafter. There was a questionable history of small amounts of orange juice at the age of 6 months. Child died of diarrhea (S. morgani) being ill for 9 days. Scurvy was not suspected clinically although beading of the ribs was recorded.

FIG. 2. No. 894. High power view of costochondral junction to show fractures which are undergoing healing in that there is a good deal of cellular (osteoblastic) activity. Osteoid is being laid down about the fractured calcified cartilage fragments. This was a 6 months old child who had been fed cow's milk all his life with never any orange juice. The terminal illness was lobular pneumonia. During the final 5 days in the hospital no vitamin C was administered. This illustrates one of the perplexing questions, i.e. evidence of healing in the absence of any pointed therapy. Scurvy was not suspected clinically although "marked beading of the ribs and flaring of the costochondral junctions" were noted.

FIG. 3. No. 1377. High power to show fragmentation of calcified matrix. There are a few cells about these spicules so that early healing is taking place. The small fragments of calcified matrix devoid of bone are, of course, abnormal. This was a 7 months old child who was fed cow's milk all his life and never received any vitamin C. He was in the hospital only 6 hours, dying of pneumococcal meningitis. Scurvy was suspected clinically because of ridged costochondral junctions.

FIG. 4. No. 1219. High power of fresh fracture of calcified matrix lattice. There is very little cellular reaction. Lighter staining material which contrasts with dark spicules of matrix is a fibrillar pink staining substance of doubtful origin. This was a 4 months old child fed on breast milk for 1 month followed by cow's milk from then on. The vitamin C history is not known as the child was dead on arrival and few data were obtained. The terminal illness was acute; lobular pneumonia was found at autopsy.

FIG. 5. No. 244. Low power of costochondral junction to illustrate an healed fracture. Such a right angled trabecula is totally abnormal. This was a 6 months old child fed breast milk for 1 month and cow's milk thereafter. The vitamin C hisotry was unknown prior to admission. The child had bacillary dysentery of 1 month's duration. He was in the hospital for 8 days, receiving 30 cc orange juice each day.

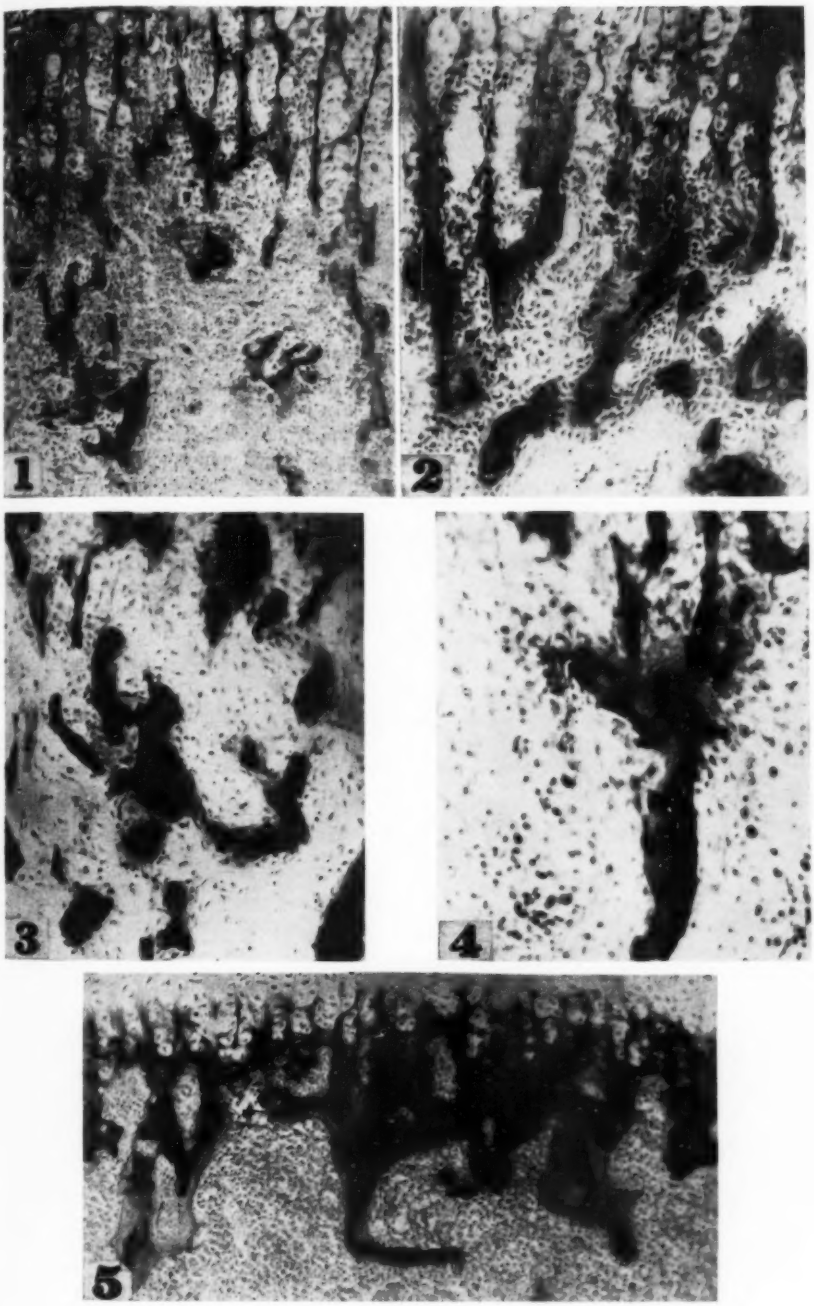
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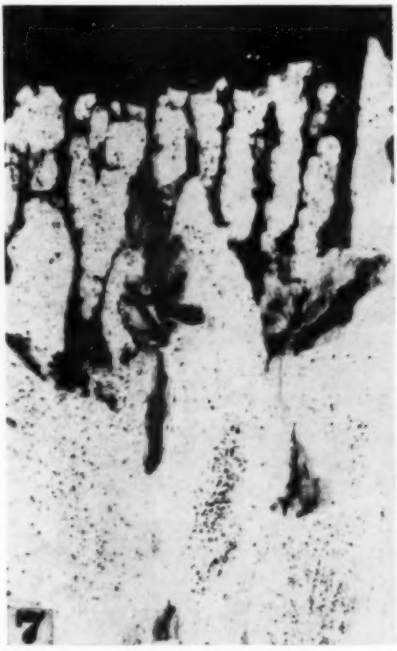
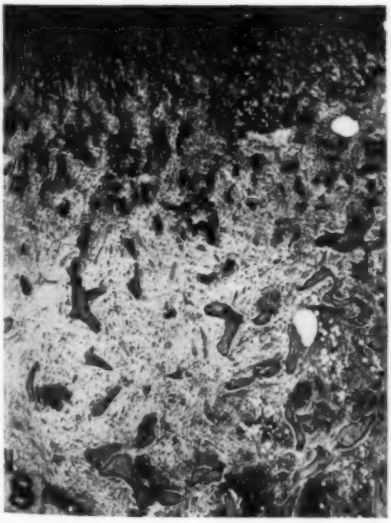
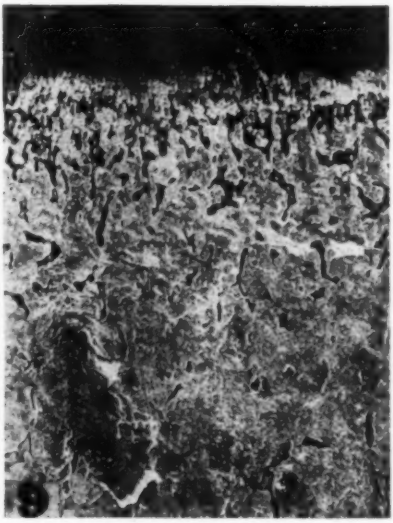
PLATE II

FIGS. 6 AND 7. No. 1219. Fig. 6. Costochondral junction to show lattice of calcified matrix in excess and devoid of any bony covering. No fractures are present nor were any found in other parts of this section which is shown to contrast with Fig. 7, the cartilage shaft junction of the lower femur where fractures are present. The two illustrate our difficulty in drawing definite conclusions regarding the presence of scurvy in the absence of fractures. Had the section shown in Fig. 7 not been available this case would have been omitted from our series.

FIGS. 8 AND 9. Nos. 1441 and 1452. There are costochondral junctions of the twins described in the text. Fig. 8 (No. 1441) was an 11 month old male who died of severe burns a few hours after reaching the hospital. The characteristic disorganized zone of fractured spicules of matrix is present beneath the cartilage. Note also the fibrous marrow devoid of myeloid elements. Fig. 9 (No. 1452) is from the twin brother who lived a month, dying of sepsis. Because scurvy was recognized in the first child at autopsy the second was intensively treated with orange juice. Now although there is a telltale stratum of abnormally appearing trabeculae beneath the cartilage all evidence of active scurvy has disappeared.







RENAL RICKETS AND OSTEITIS FIBROSA IN CHILDREN AND ADOLESCENTS

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In 1943 we (1) called attention to the rather high incidence of skeletal changes which may be encountered in adults dying of chronic renal insufficiency of one type or another. Sections from the vertebral bodies of approximately half of a series of 39 cases fullfilled the criteria of osteomalacia; that is, the trabeculae had excessive amounts of osteoid along their margins. About one third of this series presented lesions of osteitis fibrosa; that is, there were foci of bone destruction along trabeculae consisting of erosions containing osteoblasts, osteoclasts and connective tissue cells. In a given case osteomalacia or osteitis fibrosa might exist singly or together. A similar study reported by Berner (2) has confirmed our observations.

From a review (1) of the few autopsy reports of cases of renal rickets or osteitis fibrosa during the first two decades the conclusion seemed inescapable that in this group, too, one encounters disturbances in calcification with or without osteitis fibrosa. However, since certain contributions to the recent literature continue to place little emphsais on or even negate the existance of rickets, it was thought desirable to report the following series of cases. More important, however, we feel these cases provide a challenge, since the pathogenesis of the rachitic change is completely obscure. So too, although various hypotheses have been offered as to the mode of development of osteitis fibrosa, none are based on a solid foundation at this time.

CASE REPORTS

Case 1. (21959). A 32 months old white male at the time of death was first noted to be developing edema at 20 months of age. He was treated at another hospital where he was placed on a low salt diet. For several months he did well, the edema receding. However, he again became markedly swollen, was admitted to another hospital and, as the fluid continued to accumulate, was soon transferred to the Harriet Lane Home. On arrival, aged 24 months, he had and continued to have anasarca, anemia, hypertension and hypercholesterolemia. There was marked

proteinuria with numerous casts, red cells, and white cells in the sediment. During his 9 month course in the hospital the N.P.N. remained about 50 but rarely above 100 mgm.%. Serum calcium and phosphorus ranged about 7.0 to 8.0 mgm.% and 8.0 to 9.0 mgm.% respectively. CO_2 combining power averaged about 24.0 meq/L throughout the course; serum chlorides ranged about 115.0 meq/L. Serum protein was always below 4.0 gm.%, usually around 3.5 mgm.%. Albumin and globulin were on the average 1.5 and 2.0 gm.% respectively. Serum alkaline phosphatase activity ranged from 6.1 to 10.0 Bodansky units. He received many transfusions and other supportive therapy but gradually failed and died in cardiac decompensation. X-ray examination of the long bones was never carried out.

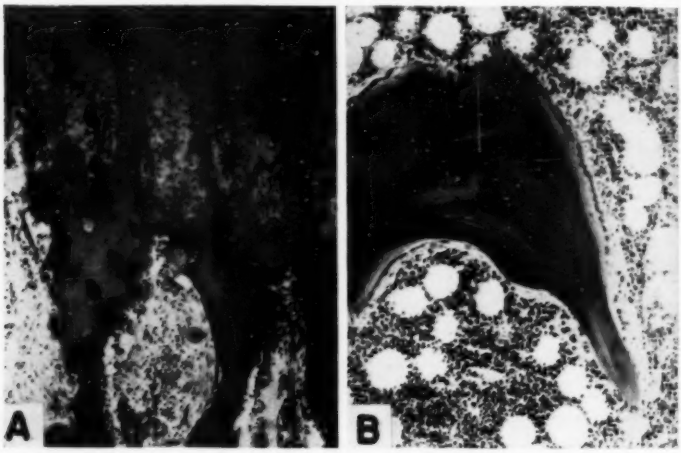

FIG. 1. Case 1. A. Costochondral junction showing decrease in width of hypertrophic cartilage cells and irregular deposition of inorganic materials (dark staining areas) in cartilage matrix. B. Trabecula in shaft to show osteoid borders which are distributed in an irregular fashion.

At autopsy the kidneys showed marked changes compatible with the diagnosis of subacute glomerulonephritis. The heart weighed 80 grams. The parathyroid glands were hyperplastic. Grossly the skeletal system was not abnormal. Microscopically (Figure 1) the costochondral junction showed marked cessation of growth of the cartilage cells. There was some irregularity in invasion of the cartilage. The undersurface was in places covered by either bone or osteoid. All had the appearance of the cartilage shaft junction in a much older individual. There was increased osteoid in the shaft, more than we have encountered in ordinary rickets at this age. There were a few foci of giant cell resorption but these were so meager that the histological changes would have to be called pure rickets.

In summary this was a case of glomerular nephritis characterized by edema, hypertension, hyperphosphatemia, hypocalcemia, hypoproteinemia but without acidosis who died of cardiac failure and in whom there was rickets but no osteitis fibrosa. There was parathyroid hyperplasia.

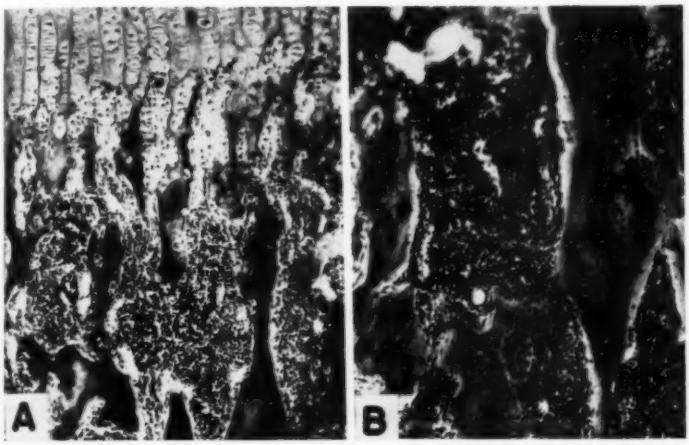

FIG. 2. Case 2. A. Costochondral junction to show defective deposition of inorganic material (dark staining) in cartilage matrix. There are osteoid borders along some of the trabeculae beneath the cartilage. B. Osteoid borders along trabeculae down in the shaft.

Case 2. (20984). A 3½ year old white male at death was first noted to have developed generalized edema at the age of 24 months. He was admitted to the hospital at this time and remained for 3 months, having an up and down course with persistent edema, hypercholesterolemia and anemia. There was hypertension, B.P. 130/86. N.P.N., 26 mgm.%; CO_2 combining power, 49.0 vol.%; calcium, 9.9 mgm.%; phosphorus 4.9 mgm.%; alkaline phosphatase, 12.0 Bodansky units; total serum protein, 3.3 gm.%; A/G ratio 0.86. He was discharged and seen in the dispensary on numerous occasions for paracenteses. During the remaining 10 months of his life the N.P.N. gradually rose to a level of 130 mgm.% three days before death. The CO_2 combining power was generally around 35 vol.%. The serum calcium gradually fell to 4.2 mgm.% a little before death while the serum phosphorus rose to a level of 15.4. The total serum protein concentration was always low ranging from 3.3 to 4.4 gm.%; the A/G ratio was usually around 1.0 but rose toward the end to 1.2. Phosphatase activity was elevated, always above 12.0

Bodansky units. He was admitted finally in convulsions and died in heart failure, blood pressure 130/110. The urine always contained protein, cells and casts. During the period of observation x-rays of the long bones showed no change.

At autopsy the kidneys showed the alterations of chronic glomerulonephritis. The heart was hypertrophied, weighing 130 grams. There was clear cell hyperplasia of the parathyroids and lobular pneumonia. Grossly the bones appeared normal. On microscopic examination, (Figure 2) the cartilage cells of the rib seemed to be proliferating in normal fashion. There was some increased destructive activity at the cartilage shaft junction, such as one may encounter in severely ill children. There was a great deal of osteoid in the shaft, but no evidence of osteitis fibrosa.

In summary this is a case of chronic glomerulonephritis of insidious onset with progressive edema, hypertension, progressive hyperphosphatemia, hypocalcemia, moderate acidosis and hypoproteinemia. There was a gradually rising N.P.N. up to 130 mgm.%. Death was thought to result from cardiac failure. Microscopically the bones showed only rickets. There was parathyroid hyperplasia.

Case 3. (6038). This case has been reported by Greene (3) in detail elsewhere. A 3½ year old white girl at the time of death had not grown well and had had numerous severe convulsions in the past. She was admitted because of fever. On examination there was obvious rickets with changes in the head, thorax and extremities. There was anemia. P.S.P. excretion in 2 hours was 7%; there were white cells in the urine. She improved enough to go home but returned and died shortly thereafter. No chemical determinations were carried out save a CO_2 combining power which 9 days before death was 19 vol.%.

At autopsy the kidneys were greatly reduced in size. There was cystic dilatation of the distal convoluted tubules together with a good deal of interstitial inflammation. The heart was not enlarged; parathyroid glands were not identified. Grossly the costochondral junctions showed marked deformation characteristic of rickets. Microscopically (Figure 3) there was irregularity in invasion with large accumulations of uncalcified cartilage. The trabeculae in the shaft had wide borders of osteoid. In addition there were islands of osteitis fibrosa throughout the shaft in which there was great disorganization of the trabeculae. Marrow cells were found aggregated in the connective tissue. There was obvious destruction of bone and osteoid as well as new formation of the latter. Rickets and osteitis fibrosa were therefore both present in this case.

In summary, this was a case of congenital malformation of the kidney in which it may be assumed that the renal insufficiency began at birth. We do not know much about the deranged chemical function

save that there was acidosis. It is a striking example of the coexistance of marked rickets and osteitis fibrosa.

Case 4. (20079). A white girl, 4 years old at the time of death was first seen at the age of 2¼ years because of generalized edema which had been present 2 months and for which she had been hospitalized elsewhere. She was under observation either in the Harriet Lane Home or dispensary during the remaining 21 months

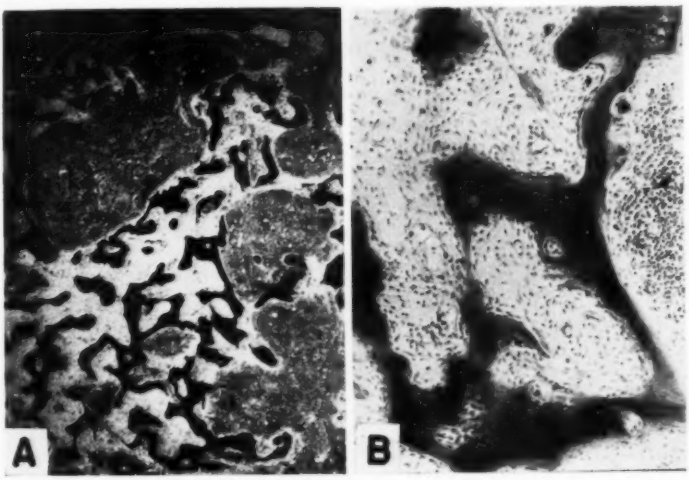

FIG. 3. Case 3. A. Low power of shaft of rib to show foci of osteitis fibrosa lying between normal marrow elements. The bony trabeculae which have formed have no orderly arrangement whatsoever. Between the trabeculae are aggregations of connective tissue cells which are shown better in the higher power. B. Here one can see the fibrous marrow and osteoclasts. The proximity of normal myeloid cells is also seen as well as borders of lighter staining osteoid along several trabeculae.

of her life. N.P.N. was continually elevated but did not rise above 100 mgm.% until 4 months before death. Total protein was always depressed, usually below 3.5 mg.%. A/G ratio was always reversed. The CO_2 combining power was always somewhat diminished. Serum calcium at the beginning was 7.3 mgm.% dropping toward the end to 4.0 mgm.%. Phosphorus on the other hand was 6.1 mgm.% on admission and rose to 16.0 mgm.% terminally. Alkaline phosphatase activity was always elevated ranging from 9.4 to 20.0 units. Cholesterol was continually elevated. The terminal event was heart failure (B.P. 120/86). X-ray examination of the extremities was never carried out.

At autopsy the kidneys were much reduced in size and microscopically showed changes characteristic of chronic glomerulonephritis. There was purulent myocarditis. The parathyroid glands were not identified. Grossly the bones showed nothing abnormal. Microscopically (Figure 4) there was irregular invasion of the cartilage with some osteoclastic destruction in this area. In the shaft of the rib and the vertebral bodies there were wide borders of osteoid about the trabeculae. In addition there were foci of osteitis fibrosa. However, the former change was out of proportion to the latter.

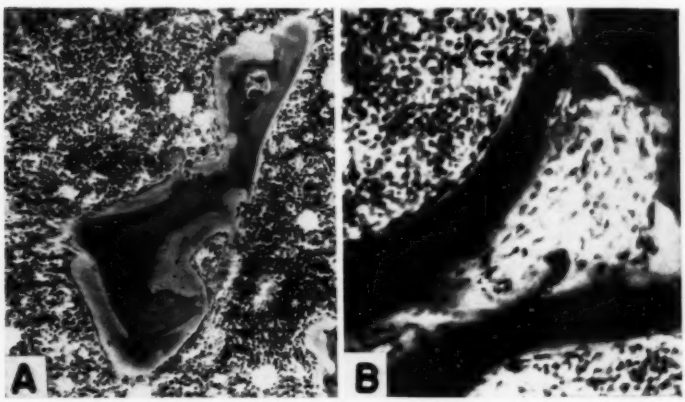

FIG. 4. Case 4. A. Trabecula from vertebral body to show borders of osteoid of varying width which practically completely encircle it. B. High power to show focus of osteitis fibrosa: connective cells and osteoclasts in an eroded zone in a trabecula.

In summary this is a case of chronic glomerular nephritis characterized by insidious onset, edema, hyperphosphatemia, hypocalcemia, hypoproteinemia, moderate acidosis and increasing hypertension, apparently resulting in cardiac failure. The bones showed both rickets and osteitis fibrosa, the former being out of proportion to the latter.

Case 5. (14388). A colored male born of syphilitic parents, 4 years old at the time of death, was found at birth to have conjunctivitis, interstitial keratitis and snuffles. After intensive treatment his STS which had been positive became negative at the age of 10 months. From this time on he grew very little and had enuresis and frequency. Six months before death he was admitted for study because of his small stature. At this time the N.P.N. was 44 mgm.%; calcium 10.5; phosphorus, 4.3; total protein, 6.4; phosphatase, 18.3 Bodansky units; P.S.P., 30%

excretion in 2 hours. X-rays of extremities showed extreme rickets. Two weeks before death he developed anorexia and complained of abdominal pain. He was brought to the hospital in convulsions. Aside from coma and convulsions there was little on examination save the chemical findings: N.P.N., 250 mgm.%; CO_2 combining power, 20 vol.%; calcium, 3.2 mgm.%; phosphorus, 24.4 mgm.%; total protein, 5.7 gm%. X-ray examination of the long bones showed rickets.

At autopsy there was chronic glomerulonephritis, parathyroid hyperplasia and lobular pneumonia. The heart was not particularly enlarged, weighing 80 grams. Grossly there was obvious rickets with swollen costochondral junctions and an irregular line of ossification. Microscopic examination (Figure 5) confirmed this

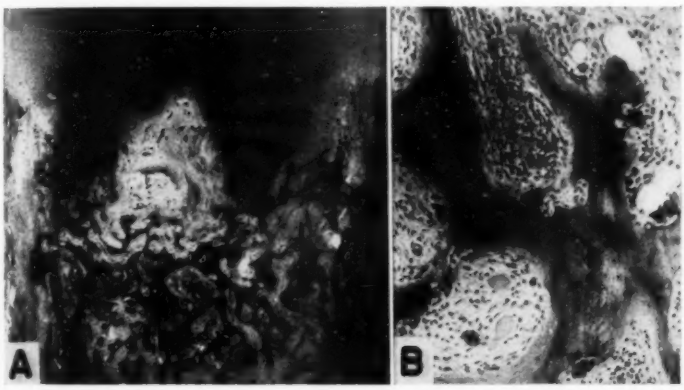

FIG. 5. Case 5. A. Low power of costochondral junction to show irregularity and defective calcification of the cartilage. Note the disorderly pattern of the trabeculae beneath the cartilage. B. Trabecula showing borders of osteoid as well as the lesions of osteitis fibrosa.

great distortion at the costochondral junction with irregular invasion and prominent tongues of cartilage jutting down into the shaft where the trabeculae were covered by broad borders of osteoid. In addition there were numerous osteoclasts present. Much of the intertrabecular space was filled with loose connective tissue; this material was found in greater quantities than were the marrow elements.

In summary this congenital syphilitic, apparently successfully treated, developed glomerular nephritis insidiously and was found to have impaired kidney function 6 months before death at which time rickets was diagnosed by x-ray; it is interesting to note that at this time serum calcium and phosphorus levels were within normal limits

although the alkaline phosphatase activity was elevated. It is likely that the renal disease progressed rapidly and he died in uremia with marked hyperphosphatemia, hypocalcemia and acidosis. There was marked rickets as well as osteitis fibrosa.

Case 6. (21380). A 5 year old girl was admitted 13 days before death with the family's complaint of "Bright's Disease". One year before she had developed pyuria which had cleared up with sulfonamide medication. The present illness had begun with puffiness of the face and the finding of proteinuria by the family physician. On examination she was underdeveloped, measuring only 36 inches in height. X-rays showed changes at the ends of the long bones characteristic of

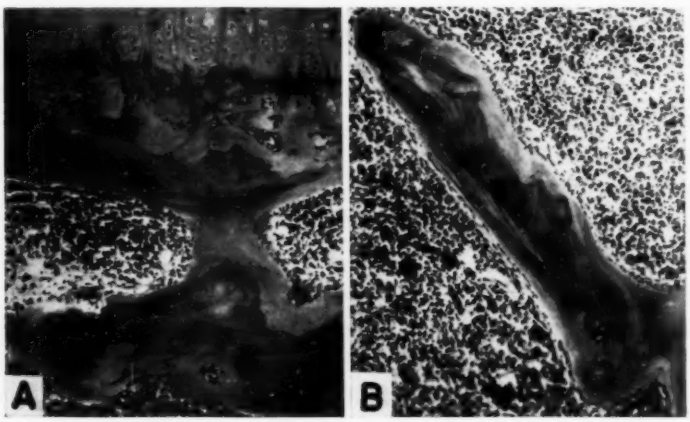

FIG. 6. Case 6. A. Cartilage shaft junction to show wide borders of osteoid with no evidence of osteitis fibrosa. B. Trabecula in shaft to again show osteoid borders.

rickets. B.P. 124/90. There was marked edema, proteinuria, numerous white cells, and a few red cells and casts. P.S.P. 5% in 2 hours. On admission the N.P.N. was 90 mgm.% falling to 53 mgm. 2 days before death. Calcium, 7.1 mgm.%; phosphorus, 5.9 mgm.%; phosphatase, 19.7 units; CO_2 combining power, 9.9 meq/L; total protein, 5.8 gm.%; A/G ratio, 0.9. Cystoscopy revealed hypertrophy of the trigone with wide ureteral orifices. A bilateral nephrostomy was performed 8 days before death. The child did poorly however, developing convulsions, cyanosis and died apparently as a result of cardiac failure with elevated blood pressure.

At autopsy there was hypertrophy of the bladder, chronic cystitis, hydroureter and hydronephrosis and chronic pyelonephritis. The heart was not enlarged. The parathyroids were enlarged. Grossly the line of ossification of the ribs was irregular and swollen. Microscopically, (Figure 6) there was irregular invasion of

the cartilage and the hypertrophic zone was increased in width. There were wide bands of osteoid about the trabeculae but no evidence of osteitis fibrosa.

In summary this child developed pyelonephritis as a result of obstruction. There was hypertension which apparently caused death, only very moderate hyperphosphatemia and hypocalcemia, but marked acidosis and some elevation of N.P.N. At autopsy there was marked rickets with no osteitis fibrosa.

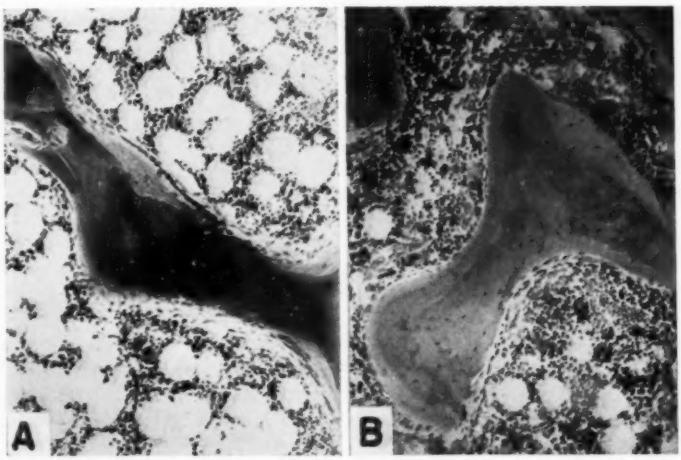

FIG. 7. Case 7. A and B. Several trabeculae in shaft of rib to show wide borders of osteoid. There are a few small foci of osteitis fibrosa. The osteoid, however, is far in excess of the latter change.

Case 7. (21846). A white girl, 6 years old at death, was admitted to the hospital 6 months before because of a skin rash and hematemesis, all of which had developed shortly after she had taken medicine for scabies. On examination she was thought to have anaphylactoid purpura. Blood study revealed reduced serum protein (3.9 gms.%), elevated serum phosphorus (7.6 mgm.%) and normal calcium, (9.7 mgm.%). There was slight acidosis. Hypertension was also noted. Because of chicken pox contact she was transferred to another hospital where she began to develop generalized edema. This persisted and she was readmitted here. During the 4 months she was under observation the N.P.N. rose to 125 mgm.%, the calcium fell to 6.6 mgm.%, the phosphorus rose to 12.0 mgm.%. Alkaline phosphatase activity was elevated. The CO_2 combining power ranged from 20 to 25 meq /L. The blood pressure rose and she died in what was thought to be cardiac failure. N.P.N. on the day of death was 125 mgm.%. X-ray examination of the long bones was never carried out.

At autospy the kidneys showed subacute glomerular nephritis. The heart was somewhat hypertrophied. The cells of the parathyroids were thought to be larger than normal. Grossly the bones showed nothing unusual. However, microscopically (Figure 7) in the shaft of the rib there were wide borders of osteoid about the trabeculae. In addition foci of osteoclastic resorption were also seen with a few connective tissue cells in the lacunae thus formed. The rickets was far out of proportion to the osteitis fibrosa, however.

In summary this is a case of rather acutely progressive glomerular nephritis with rising serum phosphorus, falling calcium, reduced protein and hypertension. The acidosis was moderate. There was marked rickets as evidenced by osteoid in the shaft with foci of osteitis fibrosa as well but the former change predominated over the latter.

Case 8. (14644). A colored male, 7 years old at the time of death was first seen at 2 years of age because of urinary frequency, incontinence, weakness and vomiting. At this time he was found to have a persistent proteinuria. At 5 years of age, following a respiratory infection he developed generalized edema and was admitted to the hospital. Here he was found to have hypertension (B.P. 180/150), cardiac hypertrophy, massive proteinuria and hematuria. At this time: N.P.N., 69 mgm.%; cholesterol, 373 mgm.%; total protein, 5.7 gm.%; A/G, 1.3; P.S.P., 55% of normal in 2 hours. The edema cleared and the blood pressure fell somewhat, so that the patient was discharged. He got along fairly well for the following year but was finally admitted 10 days before death when edema returned and he became drowsy. Blood pressure was 152/120 on admission. He had convulsions and was edematous. N.P.N., 164 mgm.% rising to 206 mgm.% on day of death; CO_2 combining power, 35 vol.% on admission; total protein, 6.0 gm.%; A/G, 1.06. No determinations of serum calcium or phosphorus were ever performed nor were x-rays of the extremities made.

At autopsy the heart was enlarged, weighing 180 grams. Both kidneys were symmetrical and small with roughened surfaces. Microscopically the kidneys showed characteristic changes of chronic glomerulonephritis. Grossly the bones showed no change. Microscopically (Figure 8) the trabeculae of the shaft were coated with broad bands of osteoid. There were foci of osteitis fibrosa along the surface of both the true bone as well as the osteoid.

In summary, there is a five year history of progressive urinary insufficiency as a result of chronic glomerular nephritis with hypertension. Unfortunately chemical studies were meagre and only indicated uremia, acidosis and normal serum protein. Morphologically, however, this case is of interest since it demonstrates the coexistance of rickets and osteitis fibrosa.

Case 9. (18837). A white boy, 11 years old at the time of death was first seen at 7 years of age because of enuresis and frequency of urination. Examination revealed that he had hypertension and the following blood chemical values: N.P.N., 45 mgm.%; total protein, 5.0 gms.%; calcium, 10.1 mgm.%; phosphorus, 3.9 mgm.%; phosphatase, 10.6 Bodansky units. He was thought to have chronic nephritis. He returned 6 months later with edema and about the same chemical findings. This time at cystoscopic examination congenital valves of the posterior urethra were thought to be present; this area was fulgurated. He was not seen again for 3 years at which time he returned with anemia and hypertension. The blood chemical values had changed as follows: N.P.N., 141 mgm.%; total protein,

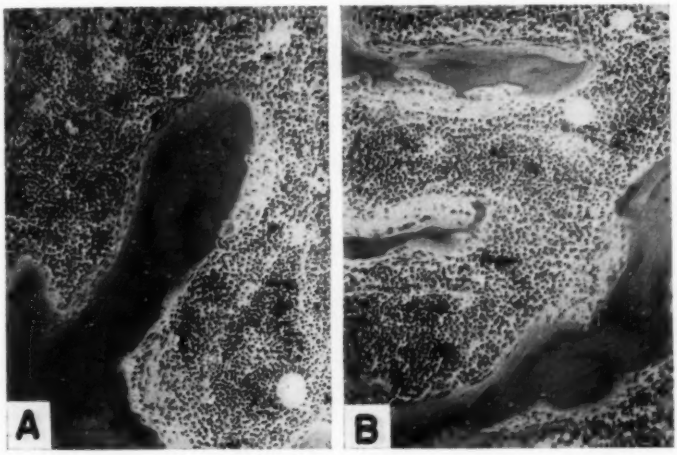

FIG. 8. Case 8. A and B. Several trabeculae from shaft of rib to show wide borders of osteoid. There are small foci of osteitis fibrosa along trabeculae. The osteoid is far in excess of the lesions of osteitis fibrosa.

6.5 gms.%; calcium, 10/1 mg.%; phosphorus, 8.2 mgm.%; phosphatase, 18.2 Bodansky units; CO_2 combining power, 46 vol.%. He was seen on several occasions during the remaining 5 months of life. The N.P.N. rose, the calcium fell to 6.1 mgm.%; the phosphorus and phosphatase activity rose to 16.8 mgm.% and 21.5 units respectively and he became more acidotic. He died in uremia. X-ray examination of the long bones 6 months before death was interpreted as normal.

At autopsy the kidneys were reduced in size and microscopically showed chronic glomerulonephritis. The heart was enlarged (250 gm.). The parathyroid glands showed hyperplasia; there was lobular pneumonia. Grossly the bones showed no change. Microscopically, (Figure 9) the trabeculae showed some increase in osteoid; more marked however were foci of osteitis fibrosa. There was

marked fibrosis of the medullary cavity with disappearance of the myeloid elements. Numerous osteoclasts were present about the trabeculae.

In summary, this child had urinary difficulties of known 4 years duration which were thought to be related to obstruction but which at autopsy were due to chronic glomerular nephritis. There was hyperphosphatemia, hypocalcemia terminally, increased N.P.N. and acidosis. Microscopically the changes in the bones were predominantly those of osteitis fibrosa although there was some rickets present as well.

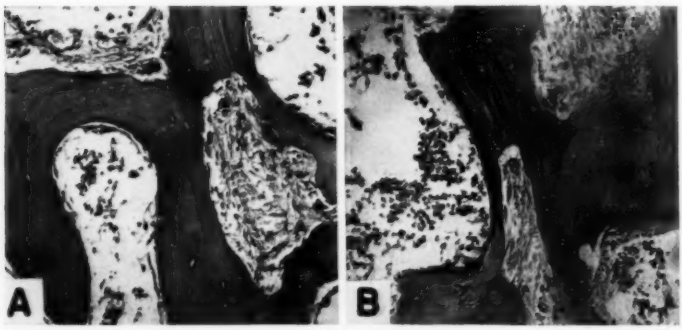

FIG. 9. Case 9. A and B. Trabeculae in shaft to show foci of connective tissue in marrow with giant cells in clefts of bone. Normal marrow elements are also seen. There are thin borders of osteoid along several trabeculae.

Case 10. (12823). A white girl, 13 years old at death, had apparently been well until 2¾ months before when her ankles became swollen and dyspnea on exertion appeared. Examination elsewhere revealed she had anemia and "Brights Disease". She became progressively weaker, anemic and edematous. On examination 19 days before death she had anemia, hypertension, retinal hemorrhages and edema. P.S.P. was zero in two hours. The following chemical determinations were performed: N.P.N., 143 mgm.%; calcium, 8.2 mgm.%; phosphorus, 10.9 mgm.%; total protein, 6.0 gms.%, CO_2 combining power, 37.7 vols. %. The urine contained many white cells and B. coli was cultured. She grew weaker and died in uremia. X-rays films of the extremities were interpreted as being normal.

At autopsy she was found to have chronic cystitis, ureteritis and pyelonephritis. There was slight generalized arteriolosclerosis; the heart was enlarged, (300 grams). The parathyroids were not found. Grossly the bones showed nothing. Microscopically (Figure 10) there was excessive osteoid about the trabeculae. In addition

many trabeculae were invested by aggregations of connective tissue cells in which there were scattered osteoclasts. The osteitis fibrosa in this case was more marked than the rickets.

In summary this was a case of chronic urinary tract infection with symptoms of only 2½ months. The changes in the kidneys, however, were much older than this. The serum phosphorus was elevated; the calcium reduced. She died in uremia. Microscopically the bones show both rickets and osteitis fibrosa but the latter predominated.

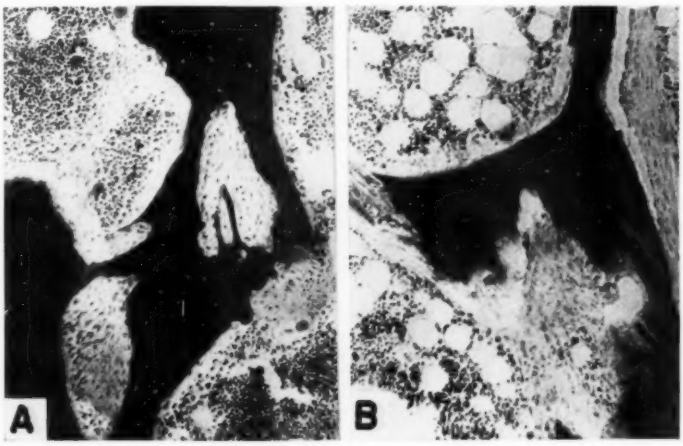

FIG. 10. Case 10. A and B. Trabeculae from shaft of rib to show pronounced changes of osteitis fibrosa. There are small areas where there are thin bands of osteoid. This change is not marked, however, in comparison to that of osteitis fibrosa.

Case 11. (8657). This case has been previously reported from this laboratory by Lathrop (4). A 15 year old white girl at the time of death was first noted to be retarded in growth at the age of 11 years. About a year later she was noted to be drowsy. She was admitted to another hospital where proteinurea, elevated N.P.N. and decrease in phenolsulfonpthalein excretion were found. During the ensuing 3 years she developed anorexia, edema and palpatation on exertion; growth seemed to have ceased entirely. On examination she appeared underdeveloped and there was anemia and edema. There was beading of the costochondral junctions, genu valgum and thickening of the wrists. During her stay of 5 weeks in the hospital her course was downward. N.P.N. rose from 100 mgm.% to 244 mgm.%. Serum

calcium fell from 5.9 to 4.6 mgm.% while the phosphorus remained about 9.0 mgm.%. Total protein was not reduced. CO_2 combining power was reduced, usually below 30 vol.%.

At autopsy the kidneys were small and scarred and microscopically showed dilated tubules, hyaline glomeruli and much interstitial inflammation. The line of ossification of the bones was irregular grossly and showed small yellowish opacities beneath. Microscopically (Figure 11) there was a great deal of loose connective tissue in the marrow spaces and was particularly accentuated about the trabeculae. There was, in addition, excessive osteoid covering these structures but the osteitis

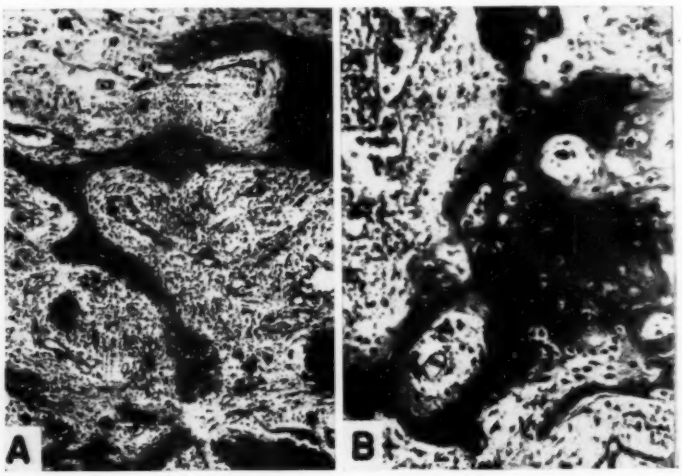

FIG. 11. Case 11. A. Low power to show fibrous connective tissue stroma in marrow with occasional giant cell. The bone is extremely cellular. B. Higher power to show osteoid borders about darker staining bony trabecula. The osteoid is very cellular.

fibrosa predominated over the rachitic change. The parathyroids were not described.

In summary this is a case of chronic renal disease with raised serum phosphorus and lowered calcium, increased N.P.N. and acidosis. The changes of osteitis fibrosa are in excess of those of rickets.

Case 12. (19608). A 15 year old boy at the time of death was first seen at the age of 2 years when he was found to have an infantile, functionless and infected right kidney. The left kidney was thought to be hypertrophied. The organ on the

right was removed. The urinary tract infection did not clear, however. Blood chemical studies were normal. He was seen from time to time in the dispensary and was readmitted to the hospital at 6 years of age with urinary tract infection and hypertension. At this time it was thought that an hypertrophied median bar was present and a punch operation was performed. He was poorly developed for his age. The blood chemical determinations showed an elevation of the N.P.N. (59 mgm.%) and a lowering of the CO_2 combining power (39 vol.%). Calcium and phosphorus concentrations were normal. He was not seen again for 7 years at which time he was admitted in uremia (N.P.N., 204 mgm.%) with edema, anemia and hypertension. He died after 2 weeks. Calcium was reduced and phosphorus elevated, being 4.7 mgm.% and 14.0 mgm.% respectively. Serum proteins were 5.5 gm.%. CO_2 combining power was reduced.

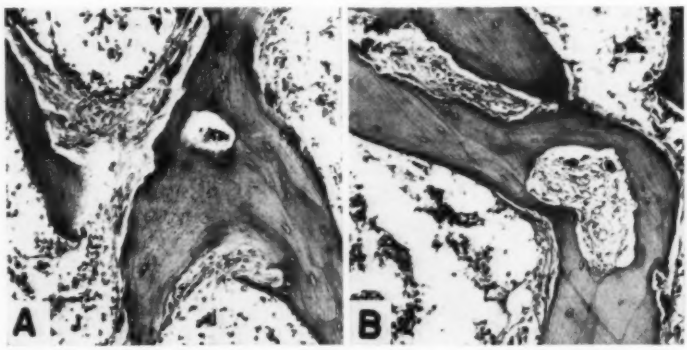

FIG. 12. Case 12. A and B. Trabeculae in shaft of rib to show foci of connective tissue proliferation with giant cells. There are thin borders of osteoid along several trabeculae.

At autopsy there was chronic cystitis and pyelonephritis, right. The heart was enlarged, weighing 220 grams. Grossly the bones showed nothing. Microscopically (Figure 12) there were thin, yet definitely increased borders of osteoid about many trabeculae. More marked were the changes of osteitis fibrosa as evidenced by aggregations of connective tissue cells and osteoclasts in defects in the trabeculae.

In summary, this was a case of long standing renal disease in one kidney since the opposite was removed at the age of 2 years. Unfortunately we know very little of the chemical changes until the end stage when the calcium was found reduced, the phosphorus elevated and uremia was present. Microscopically although some osteoid was present the predominent alteration in the bone was osteitis fibrosa.

Case 13. (12959). A 16 year old white male was admitted to the hospital for the first time 8 days before death. He was known to have had nephritis for at least 5 years. During this period he had occasional attacks of vomiting, headache, anorexia and edema. On admission he was found to have anemia, hypertension, edema and proteinuria with red and white cells. Blood chemical studies were as follows: N.P.N., 176 mgm.% rising to 200 mgm.%; CO_2 combining power 57.9 vol.%; serum calcium, 3.4 mgm.%; serum phosphorus, 14.2 mgm.%; serum protein, 5.19 gms.%; A/G, 48/52. He lapsed into coma and died.

At autopsy the kidneys showed characteristic changes of chronic glomerulonephritis. The heart was little if any enlarged (320 gms). There was early lobular

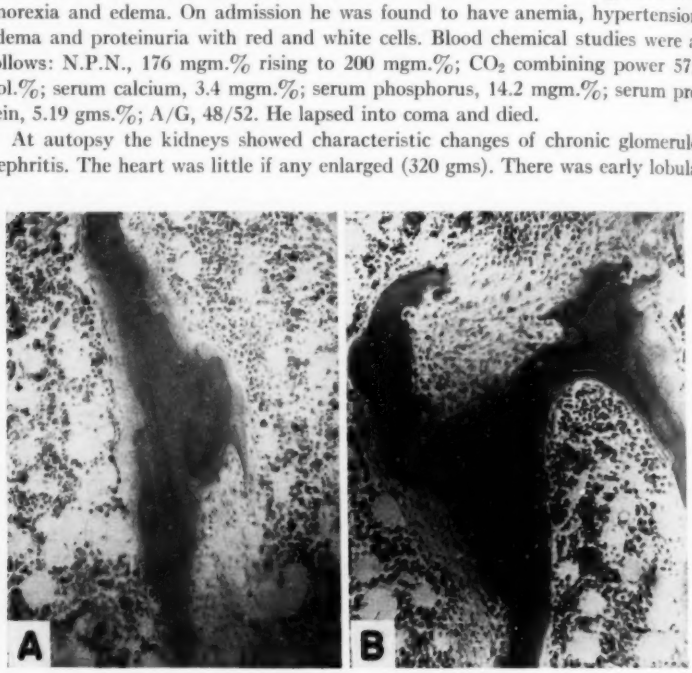

FIG. 13. Case 13. A. Trabecula from shaft of rib to show borders of osteoid. B. Another field from shaft of rib to show fibrous marrow and osteoclasts.

pneumonia. One parathyroid which was identified was enlarged. Grossly the bones showed no change. Microscopically (Figure 13) there were broad bands of osteoid about the trabeculae. There were many small trabeculae present which had a disorganized appearance. In addition there were focal erosions of the trabeculae by osteoclasts and connective tissue cells.

In summary, this is a case of chronic glomerulonephritis of at least 5 years duration. There was terminally hypocalcemia, hyperphosphatemia and slight acidosis. Microscopically the bone showed both rickets and osteitis fibrosa.

Case 14. (21311). A 16 year old white boy at the time of death was first seen 8 years before when the diagnosis of acute rheumatic fever had been made. Five years later he developed grossly bloody urine of 3 days duration and edema. The present illness in the hospital, which lasted 3 months, began with progressive sense of fatigue, nocturia, diurnal frequency and loss of weight. On examination there was anemia and hypertension, 160/92. The urine contained casts, red and white blood cells. P.S.P. excretion was zero in 2 hours. The N.P.N. was continually elevated, rising to 206 mgm.% at the end. CO_2 combining power was low, 9.9 to 6.5 meq/L. Calcium ranged from 10.3 to 8.8 mgm.%; phosphorus rose from 11.4 to 13.4 mgm.%. Total proteins were little if any lowered. He died in uremia.

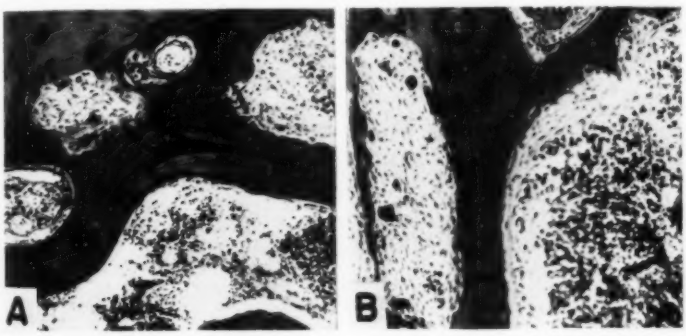

FIG. 14. Case 14. A. Costochondral junction to show foci of osteitis fibrosa as well as several trabeculae with thin borders of osteoid. B. Larger focus of osteitis fibrosa with connective tissue and giant cells. Normal marrow elements are seen close by.

At autopsy there was chronic glomerular nephritis, cardiac hypertrophy (500 gms.) and enlargement of the parathyroid glands. Grossly the bones showed no change. Microscopically (Figure 14) although there was very little osteoid there was a good deal of fibrous tissue about the trabeculae and in the medullary cavity as well. Numerous osteoclasts were found along the trabeculae.

In summary it would appear that renal disease began 3 years before and was progressive with the usual chemical changes: increased serum phosphorus, slight dimunition in serum calcium, acidosis and uremia. Microscopically there was virtually no osteoid but rather marked osteitis fibrosa.

Case 15. (20502). A 17 year old boy at the time of death first consulted a doctor 3 years before when his left leg was found to be shorter than the right. After a

cast was applied to the former the latter also became deformed. When seen a year later he appeared chronically ill with pigeon breast and marked deformities of the extremities. There was anemia but no hypertension. At this time blood chemical determinations were as follows: N.P.N., 88 mgm.%; CO_2, 16.6 meq/L; total protein, 5.9 gm.%, calcium, 8.7; phosphorus, 3.4; phosphatase activity, 22.8 Bodansky units; P.S.P. excretion, 20% in 2 hours. There was no difficulty in urination. Cystoscopic examination revealed an atonic bladder; the ureters were difficult to catheterize but one was filled with dye and x-ray revealed it to be tremendously dilated. He returned about 20 months later, having obviously become much worse. Hypertension (140/90) was present as was anemia. Blood chemical studies

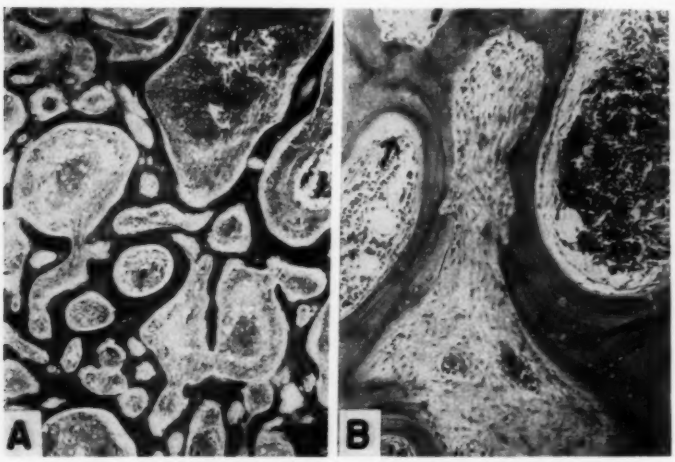

FIG. 15. Case 15. A. Low power of ileum to show totally disorganized trabeculae and connective tissue in marrow. B. High power to show predominant osteitis fibrosa; osteoid borders are also present, however (arrow).

showed: N.P.N., 121 mgm.%; CO_2 combining power, 17.9 meq/L; calcium, 5.1 mgm.%, phosphorus, 11.4 mgm.%; total protein, 5.7 gm.%. X-ray examination of the long bones revealed changes characteristic of rickets. He was discharged and returned home where he appeared to become progressively more uremic and died.

At autopsy the bladder was hypertrophied and although no obstruction could be found in the urethra, both ureters were tremendously dilated. Both kidneys were small and scarred. There was no hydronephrosis. The urinary alterations are regarded as congenital. The bones showed marked distortion grossly. Microscopically (Figure 15) the marrow spaces were replaced by a loose connective tissue in which spicules of new bone were being deposited in a totally irregular fashion. Numerous osteoclasts were found lined up along many trabeculae. One

could find trabeculae which were being destroyed on one surface and having osteoid laid down on the other. There was very little osteoid, however.

In summary this was a classic case of bone disease caused by long-standing renal insufficiency, probably congenital in origin. At the present time only the deformities marked the rachitic change, osteoid being very sparse. There was marked osteitis fibrosa.

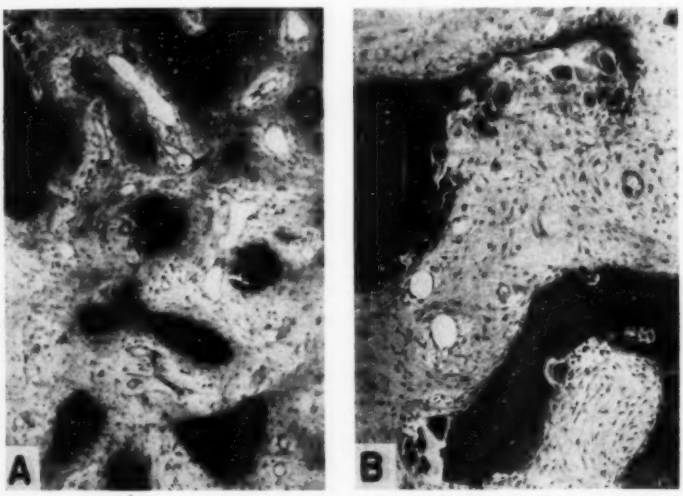

FIG. 16. Case 16. A. Low power to show abnormally appearing trabeculae and loose fibrous marrow. B. High power to show fibrous marrow and aggregations of giant cells about what appear to be several disintegrating trabeculae.

Case 16. (13571). This case has been previously reported from this laboratory by Shelling and Remsen (5). A 17 year old white boy was admitted about 5 weeks before death because of skeletal deformities and inability to walk. The abnormalities had been noted about 3 years before. There was no past history of urinary disease. Examination revealed deformities confirmed by x-ray. The peripheral vessels were hard and were shown to be calcified by x-ray. Blood chemical findings were: N.P.N., 153 mgm.%; calcium, 9.6 mgm.%; phosphorus, 8.0; serum phosphatase, 22.1 Bodansky units. Attempts to demonstrate an obstruction were unsuccessful. He gradually became more uremic and acidotic and died.

At autopsy no urethral obstruction was found; the bladder was hypertrophied as were the ureters. The kidneys showed chronic and acute pyelonephritis. All four parathyroids were tremendously enlarged. Arteries throughout the body

showed marked calcification of their walls. Grossly the bones were greatly deformed. Microscopically (Figure 16) there was marked fibrosis of the marrow with large numbers of osteoclasts lined up along the trabeculae apparently destroying these structures. Osteoblastic activity was also prominent. Little osteoid could be found.

In summary this was an instance of apparent long standing renal disease characterized by profound deformities of the ends of the bones, which at one time must have been rachitic. At this time there was little other than these to suggest rickets, osteitis fibrosa being present in almost pure form.

DISCUSSION

In the foregoing case reports we have used the terms "rickets" and "osteitis fibrosa" without defining precisely what, in our opinion, these terms connote. The fundamental disturbance in rickets in children (osteomalacia in adults) is that inorganic materials: calcium, phosphorus, carbonate, do not deposit in the organic matrix of cartilage and bone. The degree of growth of the cartilage will determine the abnormality in deposition of these materials at this site. Hence one sees the effects of rickets in the cartilage only during the first few years of life. Later, when epiphyseal growth has slowed down, inorganic deposition is able to keep up with it. If one accepts the above definition, anything which interferes with the deposition of inorganic materials in the organic matrix may be expected to produce rickets or osteomalacia. Therefore, one encounters rickets not only on a purely nutritional basis but also as a result of certain metabolic disturbances such as the de Toni-Fanconi syndrome, cystinuria, chloride acidosis, etc.

The term "osteitis fibrosa" goes back in most minds to von Recklinghausen who in 1891 (6) described a case of generalized "fibröse ostitis". For many today the term osteitis fibrosa means only hyperparathyroidism. Others feel that this term has a much wider meaning and should be used to indicate that excessive bone destruction is taking place. We tend to favor the latter view since one is able to produce the lesion by a variety of means. Therefore, in the cases reported above when the term osteitis fibrosa was used we meant only that increased bone destruction with osteoclasts, fibrous tissue proliferation, and

abnormally patterned bone were found. We do not wish to imply that hyperparathyroidism was necessarily present. It may very well have been but such has yet to be shown.

As we (1) have pointed out before the problem of the pathogenesis of rickets or osteomalacia and osteitis fibrosa is extremely complex. One need only mention the growth of bone; the intake, absorbtion and excretion of calcium and phosphorus; the presence of renal disease, its severity, duration and effect on the bone and on parathyroid stimulation; the serum protein concentration; the degree of acidosis and uremia. It would therefore seem unwise to go into a long and hypothetical discussion at this time. We have shown that rickets and osteitis fibrosa may exist singly and together. It is, we think, of interest that the former was found more often in the younger age group whose renal insufficiency had doubtless been of shorter duration. This might indicate that derangement in the calcium-phosphorus concentrations (high-P low Ca) leds to the production of a medium which does not have the power to calcify osteoid and cartilage. In collaboration with Dr. John E. Howard we are attempting to attack this problem by testing the power of nephritic sera to calcify rachitic rat cartilage. Other phases of the problem will have to be studied in animals in which renal insufficiency is produced experimentally.

The cases we have presented should make clear the morphological lesions of cartilage and bone wbich may be encountered in chronic renal disease in childhood and adolesence. Too few anatomical studies have been conducted heretofore and this doubtless accounts for the confusion which is present in the literature and the erroneous impression by many that "renal rickets" is not rickets at all but always osteitis fibrosa. For instance Peters and VanSlyke (7) state "the changes in the bones in renal dwarfism do not seem to resemble those of rickets, but rather of general inhibition of growth and development." Rule and Grollman (8) suggest that "the term "renal rickets" should be discarded. Its continued use tends to a misunderstanding of the underlying pathologic mechanisms". Bennett (9) in discussing bone lesions associated with renal rickets and renal osteitis fibrosa cystica states that "microscopically, however, there may be little if any resemblance to true rickets." Jackson et al. (10) write "renal

rickets is actually a misnomer in that the bony changes are not those characteristic of rickets and it is not even necessary that rickets be associated with this syndrome, although it is sometimes associated with rickets." Albright and Reifenstein in their recent monograph (11) say "actually the epiphyseal lesions in renal rickets under the microscope are not those of true rickets". This conclusion is apparently based on observations made on a 45 year old individual. However, in another place these authors concede that osteoid borders are present in this case but do not comment further on its pathogenesis.

One further point should be mentioned and that is the importance of the technical methods used in preparing sections. Bone should be fixed in formalin neutralized with magnesium carbonate; they should be cut in the undecalcified state after imbedding in nitrocellulose or controlled decalcification (12) should be carried out. It is our impression that the latter technique is as satisfactory as the former.

SUMMARY

We have reported a series of 16 cases of renal insufficiency in children and adolescents, 3 of the cases having been previously reported from this laboratory. Excess borders of osteoid about the trabeculae to justify the diagnosis of rickets were present in most. Changes compatible with the diagnosis of osteitis fibrosa were likewise found. The two lesions existed singly or together in a given case.

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OBSERVATIONS ON THE EFFECTS OF THE AUTONOMIC BLOCKING AGENT, BIS-TRIMETHYLAMMONIUM PENTANE DIBROMIDE (C_5) IN NORMAL SUBJECTS AND IN PATIENTS WITH PERIPHERAL VASCULAR DISEASE AND HYPERTENSION, AND COMPARISON WITH TETRAETHYLAMMONIUM CHLORIDE

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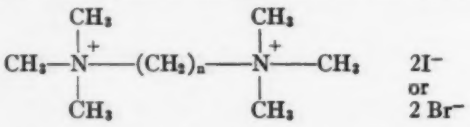
The ability of a quaternary ammonium compound, tetraethylammonium (TEA) chloride, to block the transmission of nervous impulses across autonomic ganglia (both sympathetic and parasympathetic) was demonstrated as early as 1914 by Burn and Dale (1). This action of TEA was studied by Acheson and his coworkers (2, 3) and employed to induce vasodilatation in patients with peripheral vascular disease (4), to study the various types of hypertension (5), and to reduce intestinal motility and the secretion of gastric acid (6, 7). The usefulness of TEA for these purposes has been somewhat limited by its short duration of action, by certain unexplained variations in its effects, and to a lesser extent by its side effects (4–8).

Recently, Paton and Zaimis (9) investigated the physiological effects in experimental animals of a series of polymethylene bis-trimethylammonium salts, the general formula of which is represented below:



The C_5 and C_6 derivatives of this series (n = 5 and 6) were found to have an autonomic blocking action in experimental animals that was more potent and more persistent than that of TEA (9). The effect of C_5 on the blood pressure of man was found to be similar to that of TEA, with C_5 being effective in smaller doses and more prolonged in its action (10, 11).

The studies to be reported here describe the effects of the adminis-

tration of the autonomic ganglionic blocking agent, bis-trimethylammonium pentane diiodide or dibromide (C_5), to 22 normal subjects, 16 patients with peripheral vascular disease and 25 patients with hypertension. The effects of C_5 on skin temperature, skin resistance, peripheral blood flow, blood pressure, cardiac rate, and the reflexes concerned with vasomotor tone, blood pressure, cardiac rate, and skin resistance are described, and compared with those of TEA.

METHODS

Bis-trimethylammonium pentane diiodide or dibromide (C_5) was injected intravenously in aqueous solution containing 5 mg. per ml. at a rate of approximately 5 mg. per minute in doses of 5 to 35 mg. (average 20 mg. = 0.33 mg./Kg.). There was no difference between the effects of the diiodide and dibromide salts except that the effective dose of the latter was slightly less than that of the former on the basis of weight, though it was the same on the basis of molarity, indicating that the bis-trimethylammonium pentane ion is the active group. The results recorded below for C_5 were those obtained with the dibromide salt. Tetraethylammonium chloride (TEA)[1] was injected intravenously in aqueous solution containing 100 mg. per ml. at a rate of approximately 200 mg. per minute in doses of 300 to 500 mg. (average 400 mg. = 6.67 mg./Kg.).

Skin temperature studies were carried out in a cool room (in most instances maintained at a constant temperature of 16°C.) by means of thermocouple recordings (Taylor Dermatherm) from the fingers, toes, hands, feet, arms and legs.

Blood pressure was measured by auscultation at intervals of one minute or less before and after the administration of C_5 or TEA until the maximum fall was attained, after which measurements were made at intervals of several minutes until the blood pressure returned to its original level. The subjects were recumbent, and an effort was made to insure that they were in a basal condition before any drug was administered by following the blood pressure until consistent readings were obtained. At various intervals after the injection of the drug the effect of changing from a supine to an erect position was determined by having the patient stand up. Blood pressure studies were carried out both at atmospheric temperature (21–25°C.) and in a constant temperature room at 16°C. The cold pressor test was carried out in the usual manner (12), except that the time of immersion of the hand in ice water at 4°C. was two to five minutes.

Skin resistance was determined by the resistance to the passage of a current of two microamperes from the ventral to the dorsal surface of the hand and foot (after warming the body with heat lamps), as measured by the Levine Dermometer (13). Measurement of skin resistance was made from the surface of other parts of the body with the Spartana Dermometer (14). Changes in the skin resistance of the

[1] Etamon, Parke Davis and Company.

hands and feet produced by cough, deep breathing, and electric shock (the "galvanic" reflex) were measured by the method of Richter and Whelan (15).

RESULTS

A. Effect of C_5 on Skin Temperature of the Extremities

In normal subjects: The intravenous injection of C_5 produced, in 16 out of 22 normal subjects, a definite increase in the skin temperature of the extremities (Table 1 and Fig. 1). This was, in general, somewhat greater in the lower than in the upper extremities. The increase was much greater in the fingers and toes than in the arms and legs, resulting in diminution or abolition of the temperature gradient of the extremities (Fig. 2). These changes, reflecting peripheral vasodilatation, occurred gradually over a period of 20 minutes following the injection of C_5, reaching a maximum at that time and then gradually tapering off over a period of 3 to 4 hours. They were usually accompanied by a subjective feeling of warmth, and by an increase in the blood flow through the extremities (16). In four subjects who received C_5 on successive days, there was an appreciable variation in the effect of C_5 from day to day, and occasionally the drug was without effect on skin temperature or peripheral blood flow.

TABLE 1

The effect of C_5 on the skin temperature of the extremities of normal subjects and patients with peripheral vascular disease, and comparison with TEA

SUBJECTS	COMPOUND	RISE IN SKIN TEMP. (°C.)			
		Finger		Toe	
		Range	Average	Range	Average
Normal (16)	C_5	2–6	4.6	2–10	5.2
Normal (6)	C_5	<0.5		<0.5	
Periph. Vasc. Dis. (11)	C_5	1–6	3.5	2–8	4.2
Periph. Vasc. Dis. (5)	C_5	<0.5		<0.5	
Normal (13)	TEA	1–5	2.5	2–5.5	3.5
Normal (9)	TEA	<0.5		<0.5	
Periph. Vasc. Dis. (9)	TEA	1–4	2.2	1.6	3.1
Periph. Vasc. Dis. (7)	TEA	<0.5		<0.5	

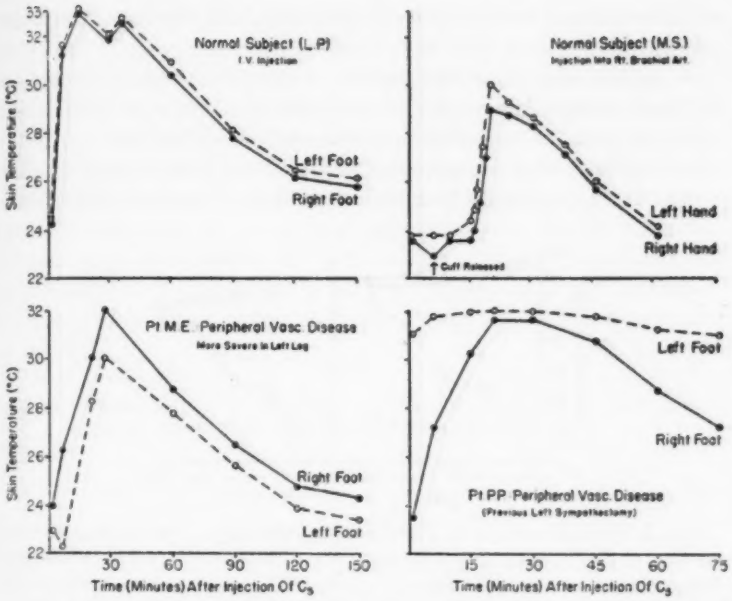


FIG. 1. The effect of the intravenous injection of 20 mg. of C_5 on the skin temperature of the lower extremities (great toe) of a normal subject (L. P.), of a patient with arteriosclerotic peripheral vascular disease (M. E.), and of a patient with arteriosclerotic peripheral vascular disease who had had a previous unilateral lumbar sympathectomy (P. P.); and the effect of the intra-arterial injection of 6 mg. of C_5 on the skin temperature of the upper extremities (middle finger) of a normal subject (M. S.).

In normal subject L. P. the skin temperature of both extremities rose rapidly. In patient M. E. it rose more slowly and to a somewhat lesser degree, especially in the more sclerotic limb. In patient P. P. there was little rise in the skin temperature of the previously sympathectomized and already warm limb, while the opposite limb increased in temperature. In normal subject M. S. there was no increase in skin temperature until the blood pressure cuff proximal to the site of the intra-arterial injection of C_5 was deflated, following which the temperature of both hands increased at approximately the same rate and to the same degree.

In hypertensive patients: C_5 produced the same effect on peripheral skin temperature and blood flow in hypertensive patients as in normal subjects. This effect was usually more marked in patients whose blood

pressure declined toward normal levels during sleep induced by sodium amytal than in those with more fixed hypertension.

In patients with peripheral vascular disease: C_5 was administered on one or more occasions to 16 patients with peripheral vascular disease, of whom 13 had arteriosclerotic peripheral vascular disease, two Raynaud's disease, and one scleroderma associated with Raynaud's phenomenon. In 11 patients (9 arteriosclerotic and two Raynaud's) there

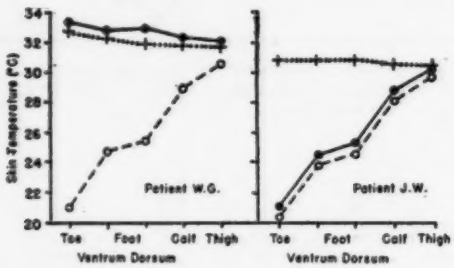


FIG. 2. The effects of C_5 and of lumbar sympathectomy on the skin temperature gradient of the right leg of two patients with arteriosclerotic peripheral vascular disease. Measurements were made in a cool (16°C.), constant temperature room before the administration of C_5 (○———○), 30 minutes after the intravenous injection of 25 mg. of C_5(●———●), and two weeks after lumbar sympathectomy (+.....+).

In one patient (W. G.) the effect of C_5 in abolishing the skin temperature gradient of the extremity was similar to the effect of sympathectomy performed later. In the other patient (J. W.), however, C_5 had no effect on the skin temerature gradient, while sympathectomy abolished it.

was a definite increase in the skin temperature of the extremities, (and in blood flow, when this was measured), while in the other five patients (four arteriosclerotic and one scleroderma) this did not occur (Table 1). Four patients with arteriosclerotic peripheral vascular disease who received C_5 on several occasions showed variations in the effect of C_5 on skin temperature similar to that observed in normal subjects. In two of the subjects whose signs and symptoms were more pronounced in one leg than in the other, the increase in skin temperature produced by C_5 consistently occurred more slowly and to a lesser degree in the leg that had the more sclerotic vessels (Fig. 1).

Comparison between the effects of C_5 and of lumbar sympathetic procaine block or sympathectomy (Table 2 and Fig. 2): Seven patients with peripheral vascular disease (five arteriosclerotic, one Raynaud's, and one scleroderma associated with Raynaud's phenomenon) who had received C_5 were later subjected to unilateral or bilateral lumbar sympathectomy. In four of these patients who had had a definite increase in skin temperature following C_5, the increase in skin temperature produced by sympathectomy was only slightly greater than the maximal increase after C_5. In the other three patients, who had had little or no increase in skin temperature after C_5, there was a much greater increase after lumbar sympathectomy. In two of the latter patients

TABLE 2

Comparison between the effects of C_5 and of lumbar sympathectomy on the skin temperature of the extremities of 7 patients with peripheral vascular disease

NO. OF PATIENTS	AVERAGE RISE IN TEMPERATURE OF TOE (°C.)	
	After C_5	After Sympathectomy
4	4.5	5.5
3	<0.5	4.8

successful lumbar sympathetic block with procaine was performed prior to sympathectomy, and a greater increase in the skin temperature of the "blocked" extremity occurred than had been observed after C_5.

When C_5 was administered to three patients with peripheral vascular disease in whom a unilateral lumbar sympathectomy had produced an increase in the skin temperature of the sympathectomized extremity with reduction of the temperature gradient, there was little further increase in the already elevated skin temperature of this extremity. The skin temperature of the opposite extremity increased as it had prior to sympathectomy (Fig. 1).

Comparison between the effects of C_5 and of TEA on the skin temperature of the extremities: This was determined in all of the 22 normal subjects and 16 patients with peripheral vascular disease. With the doses of C_5 and of TEA that were employed the average increase in the skin temperature of the hands and feet was greater after C_5 than after TEA (Table 1), and the effect was more prolonged (average of 3½ hours

compared to one hour). In addition, there seemed to be somewhat less variation in the response to C_5 than to TEA, although variations in the response to both drugs occurred in some subjects.

In the patients with peripheral vascular disease who were later subjected to sympathectomy there was a somewhat closer correlation between the effect of C_5 and of sympathectomy on skin temperature of the extremity than between the effect of TEA and sympathectomy, although the correlation, as described above, was by no means invariable.

B. Effect of C_5 on Skin Resistance

C_5 produced a slight increase in skin resistance in most subjects, the resistance to the passage of an electrical current through the hand increasing from an average of 14,000 ohms to 38,000 ohms, and through the foot from 12,000 to 34,000 ohms. In five normal subjects, however, C_5 produced no appreciable change. The average increase in skin resistance that followed the administration of C_5 was slightly greater than the average increase produced by TEA in the same subjects, but was much less than the increase in skin resistance produced by cold (250,000 ohms) or by successful lumbar sympathetic block or sympathectomy (900,000 ohms).

C. Effect of C_5 on Blood Pressure

In normal subjects and in patients with hypertension (Table 3 and Fig. 3): The intravenous injection of C_5 produced a very slight fall in blood pressure in normal subjects, a slightly greater fall in patients with mild hypertension and a greater (though usually moderate) fall in patients with moderate or severe hypertension. This was accompanied by a slight increase in the pulse rate. The fall in systolic pressure was usually more than twice as great as the fall in diastolic pressure. When the same dose of C_5 was administered to a hypertensive subject at different times, there was usually some variation in the extent of the fall in blood pressure, even when the initial blood pressures were the same. After the injection of C_5 the blood pressure gradually returned to its original level over a period of 20 to 102 minutes (average 64 minutes).

Correlation between the degree of fall in blood pressure produced by C_5

TABLE 3

The effect of C_5 on the blood pressure (mm. Hg) and cardiac rate (per minute) of normal subjects and hypertensive patients in the recumbent and erect positions, and comparison with TEA

The average duration of these effects is recorded in minutes

SUBJECTS	COMPOUND	BLOOD PRESSURE, RECUMBENT				CARDIAC RATE, RECUMBENT INCREASE AFTER C_5 OR TEA Av.	BLOOD PRESSURE, ERECT			CARDIAC RATE, ERECT INCREASE AFTER C_5 OR TEA Av.	BLOOD PRESSURE, RECUMBENT	
		Initial	Fall after C_5 or TEA				Fall after C_5 or TEA				Fall after sodium amytal	Rise during cold pressor test
		Av.	Range	Av.	Duration		Range	Av.	Duration		Av.	Av.
1. Normal (22)	C_5	129/77	−2/−2 to 36/10	16/7	38	9	12/4 to >80/50	>58/33	140	27		
2. Mild hypertension with normal renal function (6)	C_5	160/96	2/−2 to 46/24	23/12	50	9	18/8 to >100/70	>70/40	160	27	30/12	30/25
3. Moderate or severe hypertension with normal renal function (17)	C_5	203/125	6/2 to 84/42	44/18	77	15	34/10 to >130/94	>90/56	200	35	37/16	35/22
a. Fall in B.P. to or near normal after sodium amytal (11)	C_5	195/114	20/6 to 84/42	57/24	102	19	34/10 to >130/94	>104/68	220	37	44/20	40/30
b. Lesser fall in B.P. after sodium amytal (6)	C_5	216/141	6/2 to 29/10	19/8	60	8	34/10 to 88/44	64/27	140	30	24/9	26/8
4. Severe hypertension with diminished rental function (2)	C_5	236/138	90/50 and 128/59	109/54	70	4					14/0	28/2 (one patient)
1. As above	TEA	As above	0/−4 to 20/6	10/4		2	8/0 to 52/24	30/12	40	21		
2. As above	TEA	As above	2/−2 to 22/10	13/7	20	6	12/0 to 40/24	32/14	37	24	As above	As above
3. As above	TEA	As above	−4/−4 to 80/40	28/12	21	13	14/0 to 86/30	57/20	32	32	As above	As above
a. As above	TEA	As above	4/0 to 80/40	36/16	20	18	20/0 to 86/30	61/23	35	40	As above	As above
b. As above	TEA	As above	−4/−4 to 34/18	13/6	25	6	14/0 to 72/30	38/14	26	19	As above	As above

and by sodium amytal induced sleep (Table 3): This correlation was fairly good in almost all of the 25 hypertensive patients who were studied. Patients whose blood pressure fell to or near normal levels during sleep induced by sodium amytal had, in most instances, a corresponding fall after the administration of C_5. These patients also had, in general, a greater increase in blood pressure during the cold pressor test than did those whose blood pressure fell to a lesser degree following C_5 or sodium amytal.

The most striking exception to this correlation between lability of blood pressure and the degree of effect of C_5, sleep, and cold on the blood pressure was seen in two patients with marked renal insufficiency who had relatively fixed hypertension with fairly high diastolic pressure and little change during either sleep or the cold pressor test. In both patients there was consistently a striking fall in pressure after the administration of small doses of C_5 (5 and 8 mg.). There was no accompanying increase in the skin temperature of the extremities or in the skin resistance.

Comparison between the effects of C_5 and of TEA on blood pressure (Table 3): With the doses that were employed, the fall in blood pressure was invariably greater after C_5 than after TEA, and the time required for the blood pressure to return to its initial level was two and one half to five times longer. The correlation with the degree of blood pressure fall that occurred during sleep was better for C_5 than for TEA. Variations in the effect on blood pressure from day to day were somewhat less marked with C_5 than with TEA.

Correlation between the effects of C_5, TEA, and dorsolumbar sympathectomy on the blood pressure of three hypertensive patients (Table 4): Only three of the hypertensive patients in whom the effects of C_5 and TEA were studied have undergone dorsolumbar sympathectomy. All three patients had a significant fall in the supine blood pressure following the administration of C_5, TEA, or sodium amytal prior to operation, but in only one patient (M. K.) did the blood pressure reach normal limits after the administration of these compounds. All three had a marked postural hypotension following C_5 or TEA. During the first two to four weeks after the second stage of dorsolumbar sympathectomy the three patients had a reduction in the recumbent blood pressure which was quite similar to the reduction which had occurred

following C_5, TEA or sodium amytal prior to operation, and they had moderate postural hypotension. During the next nine months the supine (and erect) blood pressure of one patient (M. K.) remained consistently within normal limits, while the supine blood pressure of the other patients gradually increased to the level that had existed prior to sympathectomy. Thus, while in one patient the supine blood pressure after C_5, TEA or sodium amytal prior to sympathectomy paralleled the supine blood pressure nine months post-operatively, in

TABLE 4

Correlation between the effect of C_5, TEA, and dorsolumbar sympathectomy on the blood pressure of three hypertensive patients

PATIENT	BLOOD PRESSURE (mm. Hg)									
		After C_5		After TEA		After Sodium Amytal	Post-sympathectomy			
							Two weeks after		Nine months after	
	Initial Recumbent	Recumbent	Erect	Recumbent	Erect	Recumbent	Recumbent	Erect	Recumbent	Erect
M.K.	200/120	116/80	<70/30	120/85	<70/30	130/90	120/80	72/40	134/90	130/98
S.G.	200/124	158/108	<70/30	158/108	110/88	146/102	160/100	110/80	188/126	124/88
R.P.	220/124	165/110	<116/98	178/114	130/90	145/105	154/108	106/80	185/118	145/94

the other two patients the latter was higher. The erect blood pressure of all three patients was within normal limits nine months after operation.

D. Effect of C_5 on Reflexes that are Mediated Through the Autonomic Nervous System

The administration of C_5 was followed by the inhibition of several reflexes that are mediated in whole or in part by the autonomic nervous system, including those concerned with cardiac rate, with the maintenance of the arterial pressure in the erect position, with variations in vasomotor tone during the cold pressor test, and with changes in skin resistance.

Effect on cardiac rate: The administration of C_5 to normal or hypertensive subjects resulted in an increase in the cardiac rate of from 4 to 17 per minute. This increase is believed to be due to the inhibition by C_5 of reflexes responsible for the maintenance of vagal tone, since the

human heart is normally under predominantly vagal tone. The heart was not accelerated to the rate (approximately 130 per minute) that would result from complete vagal inhibition, but the competing influences of reduced sympathetic as well as vagal tone may have played a part in limiting the acceleration. Following the administration of sufficient atropine (2 mg. intramuscularly) to produce a cardiac rate of 130 per minute, the administration of C_5 produced no further increase in cardiac rate.

The increase in cardiac rate produced by C_5 was approximately of the same order as that produced by TEA (Table 3) but the increase persisted for a longer period after C_5 than after TEA (average of 48 compared to 20 minutes).

Effect on reflexes responsible for the maintenance of arterial pressure in the erect position: Following the administration of C_5 to normal and most hypertensive subjects, standing was almost invariably followed by a marked fall in blood pressure, to shock levels (Table 3 and Fig. 3). The fall in systolic pressure occurred more rapidly than the fall in diastolic pressure. The hypotension was preceded by an increase in cardiac rate (which probably tended to maintain the diastolic pressure by reducing stroke output) and was accompanied by giddiness, sometimes nausea, vertigo, scotomata, faintness, pallor, deep sighing respirations, and yawning. When the recumbent position was resumed there occurred, within one or two heart beats, an increase in blood pressure, usually above the level that had existed just prior to standing, and a slowing of the pulse rate, usually below the rate prior to standing. The postural hypotension and accompanying tachycardia lasted for 140 to 220 minutes, which was more than twice the duration of the decrease in the recumbent blood pressure. Small doses of C_5 often produced postural hypotension despite little or no fall in the recumbent blood pressure. The length of time that the subjects could stand before the blood pressure fell varied with the time that had elapsed since the injection of C_5 (Fig. 3).

In the hypertensive subjects whose recumbent blood pressure decreased appreciably after the administration of C_5 the postural hypotension and tachycardia were the same as in normal subjects, while in hypertensive subjects who had had little fall in recumbent blood

pressure the postural hypotension was less marked and frequently did not preclude prolonged standing without symptoms.

The postural hypotension and accompanying tachycardia that occurred after TEA were less striking and of considerably shorter duration than following C_5 (Table 3).

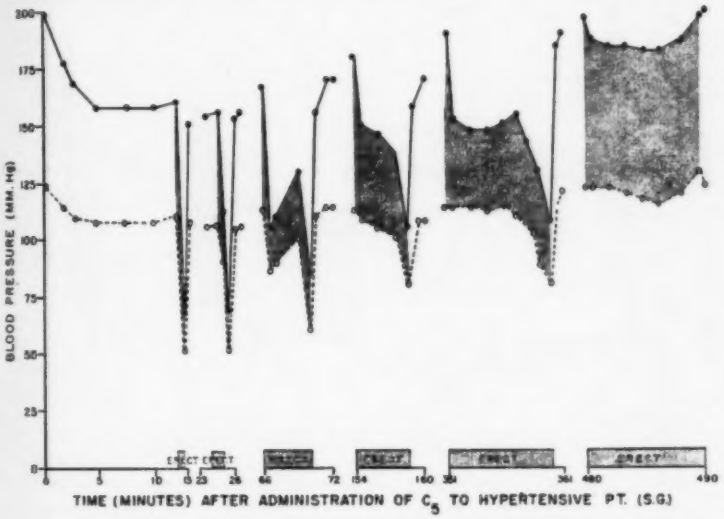


FIG. 3. The effect of C_5 (20 mg. intravenously) on the blood pressure of a hypertensive patient (S. G.). The patient was recumbent when the C_5 was injected. The shaded areas show the effect on the blood pressure of standing up at various intervals after the injection. The shaded areas represent the intervals during which the patient was erect.

Effect on reflexes responsible for the cold pressor response: The administration of C_5 inhibited to some extent the increase in blood pressure that occurred in most of the hypertensive subjects during the cold pressor test. This inhibition was usually incomplete, but was occasionally striking (Fig. 4). The inhibition of the cold pressor response following C_5 was greater and more prolonged than following TEA.

Effect on reflexes responsible for changes in skin resistance following various stimuli: Immediately following a cough, deep breath, pin prick, or electric shock, there normally occurs a transient decrease in the skin

resistance. This has been called the "galvanic" reflex (15). After the administration of C_5 there was a prompt reduction or abolition of this "galvanic reflex", occurring before any increase in skin resistance or

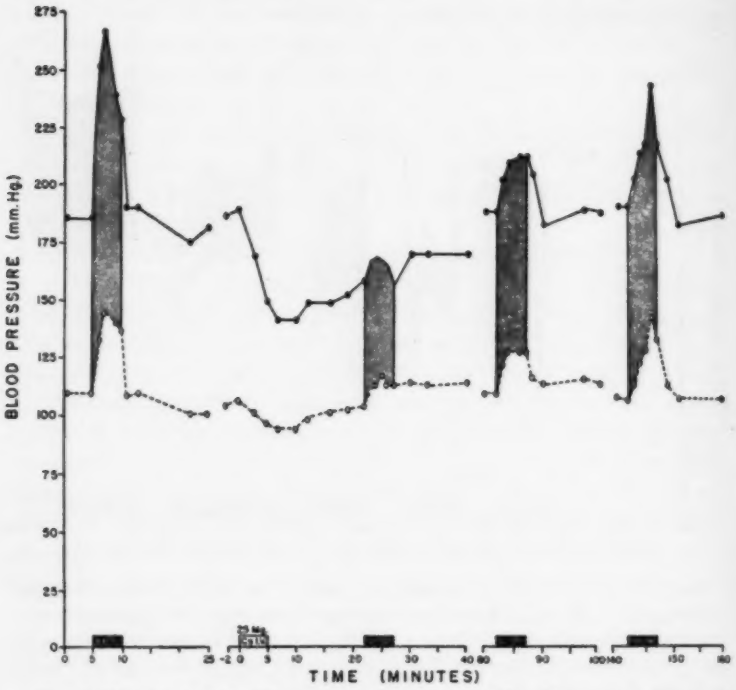


FIG. 4. The effect of C_5 (35 mg. intravenously) on the cold pressor response of a hypertensive patient (W. S.). The shaded areas represent the time of immersion (5 minutes) of the right forearm and hand in ice water.

skin temperature, and occurring even in those subjects in whom there was no increase in skin resistance.

E. Sequence of Effects of C_5 (or TEA) Following Graded Doses

The doses of C_5 (or of TEA) that were required to block the functions mediated by the autonomic nervous system varied with the different functions. The smallest doses produced postural hypotension and tachy-

cardia. Larger doses also produced a fall in the recumbent blood pressure (of hypertensive patients) with some increase in cardiac rate, peripheral vasodilatation with increased skin temperature and blood flow, and inhibition of the "galvanic" reflex. Still larger doses (e.g. 35 mg. of C_5) were required to block the cold pressor response, and the

TABLE 5

Blocking and reversal of the action of C_5 by adrenaline; lack of effect of neostigmine on the action of C_5; and lack of blocking effect of C_5 on the action of adrenaline and neostigmine

(Hypertensive patient W. S.)

The dose of C_5 was 25 mg. (i.v.), of adrenaline 0.5 mg. (i.m.), and of neostigmine 1 mg. (i.m.). The blood pressure (mm. Hg), cardiac rate (per minute), and symptoms one half hour later are recorded.

COMPOUNDS ADMINISTERED	B.P.	CARDIAC RATE	B.P.	CARDIAC RATE	SYMPTOMS
	Recumbent		Erect (2 minutes)		
None	204/118	84	200/120	96	
C_5	146/105	100	84/70	150	Giddiness when erect
Adrenaline	220/110	98	214/106	104	Slight palpitation
Neostigmine	200/108	78	192/108	90	Sweating, abdominal cramps, diarrhea
C_5 followed in 5 minutes by adrenaline	216/108	112	172/98	120	Slight palpitation
Adrenaline followed in 5 minutes by C_5	206/104	106	168/100	124	Slight palpitation
C_5 followed in 5 minutes by neostigmine	140/92	94	96/78	138	Sweating, abdominal cramps, diarrhea, giddiness when erect
Neostigmine followed in 5 minutes by C_5	154/98	92	98/80	136	Sweating, abdominal cramps, diarrhea, giddiness when erect

highest doses were needed to produce an appreciable increase in the skin resistance. The effects of C_5 (or of TEA) that were produced by small doses were, in general, of longest duration, while the effects that required larger doses were more transient.

F. Effect on C_5 Action of Adrenaline, Neostigmine, Warmth, Previous Sympathectomy, and the Intra-Arterial Route of Administration

Inhibition of C_5 action by adrenaline (Table 5): The administration of adrenaline prior to or after C_5 blocked the reduction of blood pressure,

increase in skin temperature of the extremities, and postural hypotension and tachycardia. When adrenaline was administered after C_5 the systolic blood pressure rose, indicating that C_5 had not blocked the pressor effect of adrenaline.

Lack of effect of neostigmine on C_5 action (Table 5): In contrast to adrenaline, the administration of neostigmine before or after C_5 did not antagonize the effects of the autonomic blocking action of C_5: it did not affect the degree or duration of the recumbent or postural hypotension. C_5 itself did not block the parasympatheticomimetic effects of neostigmine, such as increased sweating, abdominal cramps, diarrhea, and peripheral vasodilatation.

Inhibition of C_5 action by warmth or by previous sympathectomy (Fig. 1): When sympathetic vasoconstrictor tone was abolished by means of warmth or sympathectomy the administration of C_5 resulted in little or no further increase in the skin temperature of the already vasodilated extremity.

Lack of local effect of C_5 when administered intra-arterially (Fig. 1): The absence of any direct vasodilating action of C_5 on the blood vessels was demonstrated by injecting 6 mg. of this compound into the brachial artery. There was no immediate effect on the skin temperature of the injected extremity, and the generalized effect of the C_5 could be indefinitely delayed by occluding the venous return from the injected extremity by means of a blood pressure cuff inflated above the diastolic pressure. When the blood pressure cuff was deflated there was a gradual increase in the skin temperature of all the extremities, with reduction of the temperature gradient, similar to the effect of intravenously administered C_5. The increase in the skin temperature of the injected extremity occurred at the same rate and to the same degree as did that of the opposite extremity. There was no diminution in the grip strength of the injected extremity, indicating that C_5, in the amount that was administered, did not have neuromuscular blocking action.

G. Other Effects of C_5 and of TEA

No effects of C_5 were observed other than those deecribed above. TEA, on the other hand, produced additional effects attributable to the inhibition of parasympathetic ganglia and neuromuscular function,

and to some action on the central nervous system and perhaps on sensory receptors. Of 61 patients who received TEA, all noted a metallic taste, 42 experienced parasthesias (most marked in the hands and feet and around the mouth), 42 had moderate drowsiness, 33 had blurring of vision, difficulty of accommodation, and some mydriasis, 24 had dryness of the mouth, 11 had slight ptosis an done moderately severe ptosis, 5 had some weakness of other extraocular muscles, and one had moderate generalized weakness with dysphagia and dysarthria. Other effects of TEA which have been described, but which were not observed in these subjects, include difficulty of micturition, gastrointestinal distention, cessation of sweating, and, rarely, muscular fasciculations (4, 5).

DISCUSSION

Site and Mechanism of Action of C_6

The effects of C_6 can all be explained in terms of the blocking of functions mediated by the sympathetic nervous system, or, in the case of the increase in cardiac rate, by the parasympathetic nervous system. This blocking action of C_6 probably does not take place at the periphery of the autonomic nervous system, since C_6 does not block the action of injected adrenaline or neostigmine, and has no local effect when injected intra-arterially. The latter finding also excludes the possibility of a direct vasodilating action of C_6 on the peripheral blood vessels. There is no evidence that C_6, in the doses used, causes the formation or release of any histamine-like substance with vasodilating action, or has sufficient anticholinesterase activity in vivo to cause vasodilatation through local accumulation of acetylcholine (10). On the other hand, all the effects of C_6 are compatible with a blocking action on transmission across autonomic ganglia, which has been demonstrated in studies on the isolated superior cervical ganglion of experimental animals (9).

Since C_6 is a quaternary ammonium compound, the possibility exists that its autonomic blocking action might be due to competitive inhibition of the presumed chemical mediator of transmission across autonomic ganglia, the quaternary ammonium compound acetylcholine. However, the available evidence does not support this mechanism. Neostigmine, which increases the concentration of acetyl-

choline in autonomic ganglia (by virtue of its anticholinesterase action) does not diminish the effects of C_5. C_5 does not block the action of acetylcholine at other sites, since it does not diminish the effect of neostigmine on the gastrointestinal tract, sweat glands, and skin arterioles, nor does it reduce muscular strength when administered intra-arterially to normal subjects or to patients with myasthenia gravis (10). The autonomic blocking action of TEA, on the other hand, is antagonized by neostigmine (17), which suggests that this compound may act by the competitive inhibition of acetylcholine in the autonomic ganglia. The occurrence of muscular weakness in some individuals after the administration of TEA suggests that it may inhibit acetylcholine at the neuromuscular junction as well.

Physiological Effects of C_5

The normal peripheral vasoconstrictor tone maintained by sympathetic innervation is sufficient to produce a temperature gradient in the extremities at cool atmospheric temperature, but plays a very minor role in the maintenance of normal arterial pressure in the recumbent position (18). Thus, reduction of sympathetic vasoconstrictor tone by C_5 increased the skin temperature and blood flow in the extremities, and produced only a very slight fall in the recumbent blood pressure of normotensive subjects. In most hypertensive subjects, particularly those whose hypertension was labile and diminished during sleep, the fall in the recumbent blood pressure produced by C_5 was more marked and was accompanied by a decrease in the peripheral resistance (16). The degree of these changes appeared to depend on the contribution of sympathetic vasoconstrictor ("neurogenic") tone to the elevated blood pressure, and on the completeness of the blockade of the sympathetic ganglia. There was no evidence that the reduction in the recumbent blood pressure was due to any other cause: C_5 produced no change, or an increase, in the recumbent cardiac output (16), and no change in the electrocardiogram. C_5 did not affect the "humoral" and "intrinsic" influences on the peripheral resistance, as shown by its failure to block the action of adrenaline, and by its lack of direct effect on the vascular tree following intra-arterial administration. C_5 did produce some decrease in venous return (as reflected by reduction in the right auricular pressure), which may have been due to a decrease

in venomotor tone attributable to the blocking of autonomic ganglia. However, since this decrease in venous return was not sufficient to cause a measurable decrease in cardiac output, it could not have been responsible for the reduction in the recumbent blood pressure. The cause of the marked fall in the recumbent blood pressure that occurred in two patients with renal insufficiency and "fixed" hypertension following small doses of C_5 (Table 3) is not clear. The fall in blood pressure occurred too rapidly to suggest that delayed excretion of C_5 played a part. Other factors which may possibly enhance the hypotensive action of C_5 include cardiac failure, reduced elasticity of the arterial bed, and lowered concentration of serum sodium, but neither patient had cardiac failure, or significant arteriosclerosis (as revealed later at postmortem examination), and only one had reduced serum sodium (to 99 MEq./l.).

The postural hypotension produced by C_5 was accompanied by a reduction in stroke volume and in cardiac output (16). These changes are presumably due to the pooling of the blood in some vascular areas, attributable to the inhibition of reflex compensatory vasoconstriction which is normally initiated (through receptors in the aorta and carotid sinuses) by the transient fall in systolic pressure that follows change from vertical to horizontal stance. However, the hemodynamics of these changes are not entirely clear.

Incompleteness of the Autonomic Blocking Action of C_5 and TEA in Doses that were Administered

C_5 and TEA, in the doses that were employed, produced an incomplete block of the autonomic nervous system. In most subjects there was little or no increase in skin resistance. In some, there was little or no increase in skin temperature, and, in a few hypertensive subjects whose blood pressure declined during sodium amytal induced sleep, there was no fall in the recumbent blood pressure. In these subjects the effect of procaine block or lumbar ganglionectomy, or of sleep, demonstrated the presence of active vasoconstrictor tone. When C_5 and TEA did affect skin resistance, skin temperature, and recumbent blood pressure, there was often no correlation between the degree of these effects, and there were day to day variations. Finally, the postural hypotension was sometimes accompanied by an increase in the over-

all peripheral resistance, indicating that the sympathetic nervous system was still capable of producing vasoconstriction in some areas, and was therefore not completely blocked.

The doses of C_5 that were administered in this study were less than those recently reported by Arnold and Rosenheim (19, 20), who injected 25 to 100 mg. of C_5 diiodide intravenously over a period of 30 seconds, and slightly less than Burt and Graham (21), who injected 30 to 40 mg. over 1 to 2 minutes. These larger doses produced qualitatively similar effects to those described in this study, but doses of 40 to 100 mg. caused a greater fall in the recumbent blood pressure of most hypertensive patients than did smaller doses. The occurrence of several instances of serious fall in blood pressure led Arnold and Rosenheim to recommend that the initial intravenous dose of C_5 be not more than 40 mg. One fatality has been reported (by other investigators) following the administration of C_5, with death attributed to vasomotor collapse.

The doses of TEA that were employed in this study were approximately the same as have been used in most of the reported studies (4, 5), but the rate of administration was somewhat slower than that recommended for maximum effect on the peripheral vascular system (4). The slower rate of administration was employed in order to permit more accurate comparison with the effects of C_5, which was administered at an even slower rate than TEA, and in order to avoid the occasional serious fall in blood pressure that has been reported after the rapid injection of TEA (5, 8, 22, 23), with death in at least two instances (24, 25). The injection of C_5 and of TEA over a period of two or more minutes enabled determination of the blood pressure at intervals during administration, so that the injection could be stopped whenever the fall in blood pressure became too pronounced.

Applications and Limitations of C_5 (and TEA)

Peripheral vascular disease: When C_5 or TEA is administered to a subject who is recumbent, the resulting peripheral vasodilatation usually produces an increase in the blood flow through the extremities, even in the presence of a reduction in blood pressure that may occur if the subject is hypertensive. This action of C_5 or TEA may be employed to increase temporarily the peripheral circulation, particularly

when sympathetic vasospasm is playing a part in diminishing the circulation to the extremities. However, with the doses of C_5 and of TEA that were employed, the resulting improvement in peripheral circulation is neither as constant nor as prolonged as that which follows successful procaine block of the sympathetic ganglia or ganglionectomy. In addition, C_5 or TEA, in contrast to the latter measures, can be used only if the subject remains recumbent until the effect of the compound has entirely disappeared. If he assumes the erect position before this time, the resulting hypotension is accompanied by diminished blood flow to the extremities, and often by syncope. C_5 or TEA may be of limited value in predicting the effect of procaine sympathetic block or ganglionectomy. If either of these compounds has a definite effect on the temperature gradient and blood flow of the extremities, a similar and longer lasting effect may be expected from the latter procedures. However, if C_5 or TEA, in the doses that were employed, does not produce a change in the temperature gradient, one cannot predict that successful procaine block or ganglionectomy would likewise fail to increase the blood flow to the extremity (Fig. 2). It is possible that larger doses of these compounds may prove to more nearly duplicate the effects of the latter measures on temperature gradient and blood flow.

Hypertension: C_5, like TEA, is useful in studying the "neurogenic" component of the various types of hypertension. The doses of these compounds that were administered do not effect an invariable and complete inhibition of sympathetic vascular tone, and therefore can be used only to approximate the effect of the abolition of the "neurogenic" component of hypertension, or the effect of dorsolumbar sympathectomy. Larger doses may prove to be more useful in this regard, although in two out of three patients the supine blood pressure nine months after sympathectomy was higher than had been recorded after C_5, TEA, or sodium amytal prior to operation. Experience with much larger groups of patients has shown only a limited, and far from invariable correlation between the supine blood pressure after TEA or sodium amytal (or spinal anesthesia) and the supine blood pressure eight or more months after dorsolumbar sympathectomy (5, 8, 23). It is probable that the correlation with the effect of C_5 will also prove to be limited.

The therapeutic application of these compounds would appear to be limited to the temporary reduction of the blood pressure in situations where this would be desirable, as in the crises of hypertensive encephalopathy.

Other applications: C_5, like TEA, reduces gastrointestinal motility and the secretion of gastric acid (26). Both compounds are useful in the study of reflexes mediated through the autonomic nervous system. C_5 is capable of antagonizing to some extent the neuromuscular blocking action of bis-trimethylammonium decane dibromide (C_{10}), a compound which is employed as a muscular relaxent in electric shock therapy and during anesthesia. However, this action of C_5 is not great enough to be of practical value, particularly since the large doses of C_5 that are required may produce a fall in blood pressure and a disadvantageous inhibition of reflexes mediated by the autonomic nervous system (27).

C_5 (19, 28) and TEA (5) are effective after intramuscular as well as intravenous administration, although somewhat larger doses are required when the former route is employed. The effect of orally administered C_5 has not yet been studied in man, but in experimental animals the oral lethal dose is 10 times the parenteral dose (29). TEA is not effective when administered orally to man.

If too marked or too prolonged a fall in blood pressure occurs after the administration of C_5 or TEA, measures to be taken consist of shock position and the administration of adrenaline. The value of ephedrine and of pitressin have not yet been determined.

Advantages of C_5 over TEA

The autonomic blocking action of C_5 was three to four times as prolonged as that of TEA, and C_5 was more effective in doses that were one-twentieth that of TEA on the basis of weight, and one-fortieth on the basis of molarity. C_5 produced none of the "side effects" that occurred after the administration of TEA, and had no detectable effect on the central nervous system or on muscle strength.

SUMMARY

1. The autonomic blocking agent, C_5, administered intravenously in doses of 5 to 35 mg., increased the skin temperature of the ex-

tremities of most normal subjects and of most patients with peripheral vascular disease, but not as regularly as successful procaine block or ganglionectomy. C_5 increased the skin resistance only slightly.

2. C_5 produced a slight fall in the recumbent blood pressure of normal subjects, and a greater fall in hypertensive patients which paralleled the effect of sleep induced by sodium amytal. This action of C_5 could not be employed to predict the late effect of dorsolumbar sympathectomy.

3. C_5 inhibited several reflexes that are mediated by the autonomic nervous system, producing an increase in cardiac rate, marked postural hypotension and tachycardia, inhibition of the "galvanic reflex", and reduction in the cold pressor response.

4. Adrenaline antagonized the effects of C_5 on skin temperature and blood pressure, and was not blocked by C_5. Neostigmine did not antagonize the effects of C_5, and was not blocked by C_5.

5. The effects of C_5 can all be explained by its autonomic blocking action, which, following the doses that were administered, was incomplete. This blocking action does not appear to be the result of competitive inhibition of acetylcholine.

6. The autonomic blocking action of C_5 was three to four times as prolonged as that of TEA, and C_5 was effective in much smaller doses. C_5 produced none of the "side effects" that occurred after TEA.

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BOOK REVIEWS

Harvey Cushing: Surgeon, Author, Artist. Foreword by JOHN J. FULTON. By ELIZABETH THOMSON. 347 pp. $4.00. *Henry Schuman, Inc., New York.*

The old surgical building at the Hopkins is almost gone; in 1896 when Harvey Cushing came as Dr. Halsted's assistant resident in surgery, the Johns Hopkins Hospital was seven years old. The buildings were new, the faculty was new, and the new spirit of clinical research had already pervaded all ranks. Cushing's enthusiasms and talents were caught up in a spate of work that still, eleven years after his death, affects a widening circle of lives.

Cushing's rebellious spirit, tremendous vitality, incredible manual skill, mental precision, inventiveness, physicianship, scholarliness, artistic talent, literary facility might all have been contained in a less remarkable vessel and have become exhausted or stagnant. But he was driven and sustained by an almost impersonal realization of his capabilities, and the intensity with which he strengthened and dispersed them explains many legends which have grown about him.

Miss Thompson's book was written for the Life Science Library. She has used rich resources of biographical material with skill, discrimination, and warmth to tell the very human and often exciting story of a dedicated life. The biography was written for the general reader, but it is difficult to imagine anyone interested in the practice of medicine who will not be stirred by the story of this man.

B. G. B.

Saints, Sinners & Psychiatry. By CAMILLA ANDERSON. 206 pp. $2.95. *J. B. Lippincott Philadelphia, Pa.*

This book is not a series of psychological exposés as some may be led to believe from the title but rather a quite readable presentation of neurotic character development, its usefulness and meaning to an individual. It is well illustrated with numerous cases and utilizes a minimum of technical words and phrases. The "Saints and Sinners" of the title refers to those who are virtuous or avirtuous compulsively to avoid anxiety and not from any moral standpoint. The author sees all people as having developed character traits as an outgrowth of their attempts to gain acceptance from the significant people of their childhood, for to fail in this arouses anxiety and "fear of annihilation." Each character trait has its "entitlement" or expected reward; the combination of an individual's character traits make up his "self-image" or usual behavior patterns. The parts of the book presenting these ideas are the best; Chapter 4, "The Structure-Function Concept" is particularly well done.

However, the psychiatric reader may be rather disconcerted by some sections and statements. There are no references to the work of others, except for the statement that the author's theory has "some details in common with others' theories (Freud, Adler, Sullivan, Schilder)." Indeed much has been borrowed from them and elaboration of agreements and disagreements would be desirable. In Chapter

9, "Confusion Multiplied," at least four separate selves are introduced: They are "that we believe ourselves to be," "the vast superstructure built up as a means of getting acceptance," "the idealized self-image," and "the more comfortable and natural and realistic self"; this concept is difficult to follow, poorly elucidated and contributes little to the author's implication by emphasis that intellectual insight itself is curative (in comparison with the emotional experience in a treatment situation). The total faults of the book do not outweight its many good points so that it can well be recommended to medical students and physicians who desire to learn more about personality development.

J. M. M.

Factors Regulating Blood Pressure, Transactions of the Third Conference, May 5–6, 1949,— Edited by B. N. Zweifach and Ephraim Shorr. 280 pp., $2.55. *Josiah Macy, Jr. Foundation, New York.*

This little volume contains a great wealth of ideas concerning the dynamics of the circulation as presented, discussed, and argued by a group of the most eminent authorities in the field. The subject matter relates not only to the hypertensive state but to almost all aspects of disordered circulation. The informal nature of the conference permits the participants to exercise their full range of imaginative and critical powers. While Zweifach postulates that the capillary system through its function of regulating local flow must play a role in the retrograde regulation of blood pressure, Cournand points out that the ultimate cause of all blood pressure is the functioning left ventricle. Between the two extremes Bazett discusses the role of venous resistance; Lamport wonders about damping of the renal pulse pressure in the genesis of Goldblatt hypertension; Katz considers the effects of variations in flow distribution; and Stead leads a fascinating discussion of the dynamics of congestive cardiac failure. Grimson covers the role of the sympathetic nervous system and Kety presents his studies on cerebral blood flow. There are in addition two papers dealing with the measurement and recording of blood pressure by means of a capacitance manometer and a differential impedence recorder.

The book raises many more questions than it answers and provides ideas enough to keep many investigators at work for years to come.

A. A. K.

A Text-Book of X-ray Diagnosis, vol. 4, 2nd edition. By British Authors. Edited by S. Cochrane Shanks and Peter Kerley. 592 pp. $15.00. *W. B. Saunders Co., Philadelphia, Pa.*

This review concerns only Volume IV which is the last volume of the second edition but has been released first. The subject of this volume concerns only Bones, Joints, and Soft Tissues. The first edition originally was composed of three volumes. The addition of much new material and an expansion of the previous text necessitated a separate volume to cover this subject. Apparently the four volumes have been planned to be used as separate, specialized texts or together as a complete treatise on general radiology.

Most students of radiology are quite familiar with the first edition. It is generally regarded as one of the two best comprehensive treatises of X-ray diagnosis in the English literature. Despite the death of their colleague Dr. Twining, Drs. Kerley and Shanks have done a superb job of editing this volume.

The book is divided into eleven parts as follows: 1– Normal Bones and Joints, 2– Pathology, 3– Congenital Deformities, 4– Trauma, 5– Inflammatory Diseases, 6– Osteochrondritis, 7– Static and Paralytic Lesions, 8– Constitutional Diseases, 9– Tumors, 10– Soft Tissues, and 11– Foreign Bodies.

These subjects are thoroughly discussed and well illustrated by twelve of the seventeen eminent British medical authorities contributing to the entire work. After each part is appended a valuable subject reference. The part on Bone Pathology has been revised to a certain extent. The part dealing with Trauma has been greatly enlarged and completely rewritten. The section devoted to Inflammatory Diseases, particularly tuberculosis, syphilis, and uncommon inflammatory diseases has also been extensively revised. These two parts deserve special commendation. In Part 7, Static and Paralytic Lesions, a chapter on spondylolisthesis has been added. In Part 8 on Constitutional Diseases several paragraphs have been added on the Fanconi syndrome and also on fluorine poisoning. Part 9, Tumors and Cysts, has been basically reorganized and considerably expanded. Entities such as osteoid-osteoma and Mafucci's syndrome have been added. In Part 10, Soft Tissues, paragraphs have been added on calcifications seen in Ehler Danlos syndrome, the parasite Armillifer Armillatis, and several new interesting paragraphs on edema and bacteriogenic gas in soft tissue. A concept of the expansion can be gained by the fact that there are 148 more pages concerning Bones, Joints, and Soft Tissues in this edition than in the previous edition.

The only aspect which may be disconcerting to the average American reader is the reproduction of the roentgenograms as positives rather than in the original negative density scale. The printing and quality of materials used in this book are excellent. This work merits unqualified approval and is now more than ever capable of serving as a modern, standard, advanced general text of X-ray diagnosis.

D. M. G.

Delayed Union in Fractures of the Long Bone. By KENNETH W. STARR. 215 pp. $9.00. *The C. V. Mosby Co., St. Louis, Missouri.*

This book, awarded the Jacksonian Prize by The Royal College of Surgeons of England in 1944, was published in 1947. It is interestingly written, contains many historical references and is punctuated by many quotations and aphorisms.

The body of the Essay begins with a section on "The Morphogenesis of Bone," in which there is well presented the facts known about the microscopic structure of bone and its chemistry as well as a consideration of bone physiology insofar as it deals with bone deposition, bone resorption, and the relationship of function to the shape and structure of bone. Well-chosen and well-reproduced illustrations help make this a very readable survey. The next section is devoted to "The Healing of Fractures." In it the author brings us up-to-date on the studies that have

been published on the various tissues involved in the fracture process and their role in the repair of the fracture. The consideration of the "Aetiology and Treatment of Delayed Union" is left for the final third of the book. After an enumeration of the factors generally held responsible for the delay in fracture healing, a short statistical report is given, based on a survey of one thousand fractures treated in the hospital. There follows a consideration of the principles concerned in the treatment of the patient and of his fractures, simple and compound. The desirability of assisting the circulation is stressed. The author "advocates, without qualification, a new attitude to the pathological problem of delay in union of fractures," and insists "that the traumatic surgeon should abandon the mechanical for the biological, and laudable pus for scientific chemotherapy." In spite of all this, he feels, quite properly, that "it would be idle to suggest that the riddle [of delayed union] is solved."

I. W. N.

Pathology in General Surgery. By PAUL W. SCHAFER. 581 pp. $17.50. *The University of Chicago Press, Chicago, Illinois.*

This is a delightful book to read and to own. The author, who is Professor of Surgery at the University of Kansas School of Medicine, is to be warmly congratulated on an excellent piece of work. As stated in the Preface, "An effort has been made to present general surgical diseases systematically with illustrations of the most commonly encountered conditions, together with a recent bibliography which has been selected as a guide for the reader who wishes to obtain more complete information." The subject matter is arranged logically by organ systems; congenital anomalies, inflammatory lesions and neoplasms are covered in sequence. Each disease entity is presented in a concise but thorough description with emphasis on correlation of clinical and pathological features. Physiologic disturbances, diagnostic procedures and rational modes of treatment are briefly discussed to complete the coverage of each entity which is described. Beautiful color plates—368 in all—depicting gross and microscopic features of the abnormalities described in the text add immeasurably to the fund of information contained in the book. Appropriate use is also made of roentgenograms and photographs of operative fields.

Pathology in General Surgery is written largely from the clinical point of view and in consequence possesses a dynamic quality which is often lacking in textbooks of surgical pathology. The arrangement of the material by organ systems and an adequate index make this an excellent "quick-reference" volume. Dr. Schafer's book is to be highly recommended as a valuable addition to the library of the student, the surgeon and the pathologist.

H. W. S., JR.

World Surgery. By STEPHEN A. ZIEMAN. 181 pp. $6.00. *J. B. Lippincott Co., Philadelphia, Pa.*

This is a volume of abstracts of articles "from surgical journals of practically every nation and every language" published during 1949. The author is Abstract

Editor of the Journal of the International College of Surgeons, and an abstractor for International Abstracts of Surgery. The abstracts are arranged in sections: gastrointestinal, cardiovascular–respiratory, gynecologic, genito-urinary, etc.

The book shares with all abstract publications a major fault—there is rarely enough material in the abstract to satisfy the reader, often too little to help him decide whether to read the original article. In addition, there are surprising grammatical errors, and even an illustration which shows an entirely different procedure from that described in text and legend. Also, granting that the author of a group of abstracts has a problem in selecting the most important journals and articles for condensation, a singular lack of discrimination seems to have been displayed when Malayan, Panamanian and Egyptian journals are abstracted, but the Journal of Thoracic Surgery, for example, is omitted.

In sum, it is difficult to conceive of any usefulness for this publication.

R. C. C.

Histology. By ARTHUR WORTH HAM. 756 pp. $10.00. *J. B. Lippincott Co., Philadelphia, Pennsylvania.*

The author is to be commended highly for this fine new text which will certainly mark an important post in the history of medical education. I have studied the book with great interest. Arthur Ham is an impressive teacher with a wide experience in active research. His book is a remarkable adventure in pedagogy, in which he presents in a fascinatingly effective way the applications of histology in physiology and the practice of medicine. Professor Ham addresses his book chiefly to the medical student, but any graduate student would profit by reading his lucid account of the functional aspect of histology. I predict that the book will develop in future editions into a much needed Histological Basis of Physiology and Medicine.

However, in its present form, the book seems unintentionally to emphasize not the fundamental principles of histology, but rather the physiology and pathology of tissues. It does not appear to be advisable in most medical schools (most of whose students have had very little prerequisite biology) to teach histology so that it embraces primarily only those aspects which deal with physiology and disease. The text should be expanded, even at greater monetary expense, to include in fine print its valuable relation to biology and medicine in clear unmistakable terms to avoid the danger of giving the student a premature or incomplete conception of the medical aspects of histology. Professor Ham has prepared an admirable text that avoids spoon-feeding the student with summaries of desiccated histological data in the gold-labeled crystal vials that are generally used for this purpose,—he has not allowed himself sufficient pagination in the book. The exceedingly valuable part of the text on histophysiology and histopathology should be presented in fine type in order to give more space for the normal histology and for expansion of the author's purpose.

The major purpose of Chapter 5 on Cells is to give the student a foundation for his anticipated study of diseased tissues, notably tumors. It should be noted that

one of the most conspicuous problems in the many fields of cancer research is the dearth of scientists who have had a thorough training in the anatomy and physiology of normal cells. The photomicrograph of cancer of the breast is not very typical of this well-known mouse tumor, and the inquisitive medical student will surely wonder at once whether tumors of human beings have similar properties and appearance. It would have been perhaps better to substitute a basal-cell or squamous-cell carcinoma of human skin. The important subjects of cell potency and differentiation are discussed. An excelling chapter on bone, including 15 pages with beautiful photo-micrographs of abnormal bone, is devoted mainly to the pathological and clinical aspects of the subject,—while in the chapter on endocrine glands, for example, space evidently limits the discussion of the thyroid gland to scarcely more than one page of text on normal structure with one photomicrograph from the gland of a normal dog. Space did not permit any illustration of human thyroid gland or of the gland in different physiological states. The remaining six pages of the division on the thyroid gland are taken up by the equally important histophysiology, pathology and clinical aspects of this gland,—and of this part one whole page is utilized by an elegant highly magnified photomicrograph showing radiographs of the glands of rats (fed excess iodine) for which there is given no comparative photomicrograph of the normal gland. It is doubtful whether very many medical students, even in our best schools, are prepared to appreciate fully this valuable information without a basic knowledge of the detailed structure of the normal thyroid gland. On the other hand, the wisely included accounts of the physiology and pathology of the thyroid gland are necessarily incomplete in limited space. In another chapter, skin grafting, blisters and the histological basis of edema are explained in clear accurate style,—but in those pages are given also the detailed histology of skin from normal individuals and the structure and function of the capillaries of young and old. The structure and function of the Purkinje system of the heart are given, with a timely and much appreciated discussion of the elements of electrocardiography which whets the curiosity of the reader and leaves him wishing that the author had told him more in fine print.

The histology of the central nervous system is unusually brief on the debatably justifiable assumption that this subject is best taught and generally given in a separate course in neuroanatomy. It is so briefly touched upon that it is scarcely of much real value. Professor Ham feels that the peripheral nervous system, however, with its nerve endings is still the property of the general histologist. Six pages are devoted to the extensive subject of the peripheral nervous system (including degeneration and regeneration), and two pages and several diagrams to receptors concerned in cutaneous and deep sensibility. Specimens of nerve endings in skin are rarely studied in neurology, and the reader would profit by a discussion of a composite diagram of innervation of human skin such as Woollard, *et al.*, have shown. The Bayliss and Starling "law of the intestine" is well explained, but it would be difficult from the text for a beginning student to recognize and evaluate the cell-bodies of ganglionic neurons in the plexus of Auerbach or the plexus of

Meissner, or those in the renal plexus, pancreas, seminal vesicle, medulla of the adrenal gland, and elsewhere.

The book contains many extraordinarily helpful three-dimensional diagrams finely executed by one of the best artists,—although the photomicrographs are generally not too good either from the point of view of showing the student what is depicted by diagram or of printing reproduction. The tissues of the liver, pituitary gland, kidney, lung, parotid gland, small intestine, retina, stomach, testis, pancreas, and other organs are described without benefit of many photographs. The reticuloendothelial system and connective tissue are not very extensively illustrated. The chapter on cells has too few clear photomicrographs, and the student without experience with mitochondria will wonder whether the minute bodies actually exist at all. The stages of mitosis are shown in the endometrium of a rat that had been treated with a large dose of female sex hormone (presumably estrogen). Reticulocytes are shown in a rat regenerating new blood (probably after induced hemorrhage). Since the author is writing chiefly to medical students, it would be more impressive to show reticulocytes of human beings following exposure to high altitude (mountain climbers and decompression chambers). This would provide an opportunity to include a more detailed account of caisson disease, oxygen-carrying capacity, ovulocytosis, and other related aspects of low barometric pressure phenomena, all of which could be put in fine print. The new terminology in hematology is most valuable.

Although collagenic fibers are clearly and extensively described in the text, there is no good illustration of these important structures. Most enlightening dissertations are given on hyaluronidase and on such things as heparin in mast cells. The student would profit by similar discussions of fat metabolism, fatty metamorphosis, and fat infiltration of the liver. It would be helpful to discuss glycogen more fully. The mechanism of glycogenolysis is discussed, and the reader would expect a fine-print description of such matters as von Gierke's disease and storage of glycogen in the liver of the fetus and new-born infant, judging by the general purpose of the whole book. Glycogen in the fetal liver is notoriously resistant to release by adrenalin, and this book would quite justifiably include such physiological data. Few teachers are as well qualified as Professor Ham to write such a text. The reader will probably be disappointed in finding too brief reference to the regenerative capacity and the development of the liver, and to hepatoma. Subjects such as the juxtaglomerular apparatus and the regional functional differences in the organ of Corti (Wever and Bray; Guild), are only lightly touched.

The text is printed in clear easily readable type on excellent paper. The general format and the nature of the material would lend themselves better to single-column print than to double-column print from the point of easy reading, although the double column is admittedly more economical. A good feature is the distinct running-title in bold-face type at the top of each page. The reproduction of photomicrographs appears to have been done with not fine enough screen. The carefully sub-headed and inclusive index in three-column form (six columns on two facing pages) will be much appreciated by busy medical students. The book is unique

for its thoughtful organization and classification of subject matter, and the inclusion in it of most helpful pointers about techniques and artifacts. The reader may wish, however, that the author had discussed more of such facts as the vacuolated appearance of glycogen in the liver of a person who died suddenly by accident as compared to one from the ordinary hospital autopsy which is depleted of glycogen because the patient had eaten little preceding death. Although there are many things to be desired in this first edition of a text on physiological histology for medical students, it is hoped that such an important foundation in a neglected aspect of teaching histology will be expanded quickly in future editions into a book of perhaps two-fold length. Arthur Ham is an eminent leader in this serious undertaking, and his book shows evidence on every page of long and thoughtful planning and of a thorough knowledge of diverse fields of difficult research. The author should not sacrifice histology for pages and economical space and price of books. Histologists should take a bold stand in this respect, just as physiologists and pathologists and biochemists and clinicians have demanded not only big thick books but also strict prerequisites. The development of this new book will be watched and studied with intense interest because it presents histology in its proper role as the basis of normal and pathologic function and as the very foundation of biology and medicine.

Ross C. MacCardle

BOOKS RECEIVED FOR REVIEW

An Atlas of Human Anatomy. By BARRY K. ANSON. 518 pp. $11.50. *W. B. Saunders Co., Philadelphia, Pa.*

Biological Oxidation of Aromatic Rings (Biochemical Society Symposia No. 5). Edited by R. T. WILLIAMS. 96 pp. $2.00. *Cambridge University Press, New York.*

Cell Physiology and Pharmacology. By J. F. DANIELLI. 156 pp. $3.00. *Elsevier Pub. Co., New York.*

Enzymes—Growth and Cancer. By VAN R. POTTER. 64 pp. $1.85. *Charles C. Thomas, Springfield, Ill.*

Essentials of Medicine, 16th edition. By CHARLES PHILLIPS EMERSON, JR., and JANE ELIZABETH TAYLOR. 815 pp. $4.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Eyes and Industry, 2nd edition. By HEDWIG S. KUHN. 378 pp. $8.50. *The C. V. Mosby Co., St. Louis, Missouri.*

Freud. Dictionary of Psychoanalysis. Edited by NANDOR FODOR and FRANK GAYNOR. With a Preface by Theodor Reik. 208 pp. $3.75. *Philosophical Library, New York.*

Immortal Magyar: Semmelweis, Conqueror of Childbed Fever. By FRANK G. SLAUGHTER. 211 pp. $3.50 *Henry Schuman, Inc., New York.*

McClung's Handbook of Microscopical Technique for Workers in Animal and Plant Tissues, 3rd edition. Edited by RUTH MCCLUNG JONES. 790 pp. $12.00. *Paul B. Hoeber, New York.*

Nursing in Prevention and Control of Tuberculosis. By H. W. HETHERINGTON and FANNIE W. ESHLEMAN. 361 pp. $4.50. *G. E. Putnam's Sons, New York.*

Pathologic Physiology: Mechanisms of Disease. Edited by WILLIAM A. SODEMAN. 808 pp. $11.50. *W. B. Saunders Co., Philadelphia, Pa.*

Physiology and Anatomy. By ESTHER M. GREISHEIMER. 841 pp. $4.00 *J. B. Lippincott Co., Philadelphia, Pa.*

Plastic and Reconstructive Surgery. By FERRIS SMITH. 895 pp. $15.00. *W. B. Saunders Co., Philadelphia, Pa.*

Post-Graduate Lectures on Orthopedic Diagnosis and Indications. Vol. 1. By ARTHUR STEINDLER. 302 pp. $7.50. *Charles C. Thomas, Springfield, Ill.*

Practical Gynecology. By WALTER J. REICH and MITCHELL J. NECHTOW. 449 pp. $10.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Principles of Public Health Administration. By JOHN L. HANLON. 506 pp. $6.00. *The C. V. Mosby Co., St. Louis, Missouri.*

Reports on Biological Standards: VI. The Design of Toxicity Tests. By W. L. M. PERRY. 51 pp. 1s. 6d. net. *His Majesty's Stationery Office, London.*

Researches on the Radiotherapy of Oral Cancer. By CONSTANCE A. P. WOOD and J. W. BOAG (with P. Howard-Flanders, A. Glucksmann, L. H. Gray and F. G. Spear). 148 pp. 12s. 6d. net. *His Majesty's Stationery Office, London.*

Surgery of Cataract. By DANIEL B. KIRBY. 695 pp. $30.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Surgical Nursing, 9th edition. By ELDRIDGE L. ELIASON, L. K. FERGUSON, and LILLIAN A. SHOLTIS. 728 pp. $4.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Techniques in British Surgery. Edited by RODNEY MAINGOT. 733 pp. $15.00. *W. B. Saunders Co., Philadelphia, Pa.*

Textbook of Abnormal Psychology. By ROY M. DORCUS and G. WILSON SHAFFER. 717 pp. $5.00. *Williams & Wilkins Co., Baltimore, Md.*

A Text-book of X-ray Diagnosis, vol. 4, 2nd edition. By British Authors. Edited by S. COCHRANE SHANKS and PETER KERLEY. 592 pp. $15.00. *W. B. Saunders Co., Philadelphia, Pa.*

Thoracic Surgery. By RICHARD H. SWEET. 345 pp. $10.00. *W. B. Saunders Co., Philadelphia, Pa.*

The Truth About Your Eyes. By DERRICK VAIL. 180 pp. $2.50. *Farrar, Straus & Co., New York.*

Tumors of the Head and Neck. By GRANT E. WARD and JAMES W. HENDRICK. 832 pp. $15.00. *Williams & Wilkins Co., Baltimore, Md.*

Williams Obstetrics, 10th edition. By NICHOLSON J. EASTMAN. 1200 pp. $12.50. *Appleton-Century-Crofts, Inc., New York.*

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